IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BOSTON SCIENTIFIC CORPORATION and BOSTON SCIENTIFIC SCIMED, INC., Plaintiffs/Counter-Defendants, v. JOHNSON & JOHNSON, CORDIS CORPORATION, and WYETH Defendants/ Counter-Plaintiffs.	**REDACTED PUBLIC VERSION** Civil Action No. 07-333-SLR Civil Action No. 07-348-SLR Civil Action No. 07-409-SLR
BOSTON SCIENTIFIC CORPORATION and BOSTON SCIENTIFIC SCIMED, INC., Plaintiffs/Counter-Defendants, v. JOHNSON & JOHNSON, CORDIS CORPORATION, and WYETH Defendants/Counter-Plaintiffs.	Civil Action No. 07-765-SLR

APPENDIX OF EXHIBITS TO THE RESPONSE BRIEF OF JOHNSON & JOHNSON, CORDIS CORPORATION, AND WYETH IN OPPOSITION TO PLAINTIFFS' MOTION FOR SUMMARY JUDGMENT OF INVALIDITY OF U.S. PATENT NOS. 7,217,286, 7,223,286, 7,229,473, AND 7,300,662 UNDER 35 U.S.C. § 112

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United States Patent [19]

Skotnicki et al.

[11] **Patent Number: 5,362,718**

[45] **Date of Patent: Nov. 8, 1994**

[54] **RAPAMYCIN HYDROXYESTERS**

[75] Inventors: **Jerauld S. Skotnicki**, Allentown; **Christina L. Leone**, Princeton, both of N.J.; **Guy A. Schiehser**, Yardley, Pa.

[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[21] Appl. No.: **229,261**

[22] Filed: **Apr. 18, 1994**

[51] **Int. Cl.**[5] **A61K 31/695**; A61K 31/395; C07D 498/16; C07D 7/04

[52] **U.S. Cl.** **514/63**; 514/291; 540/452; 540/456

[58] **Field of Search** 540/456, 452; 514/291, 514/63

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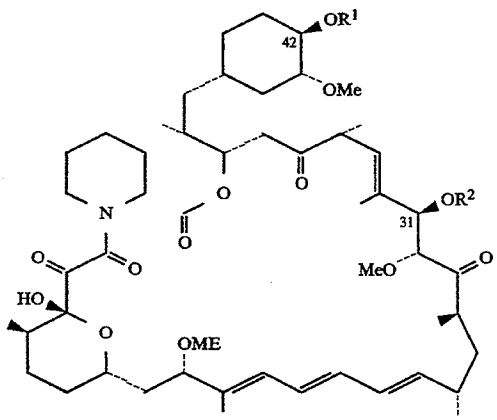
[57] ABSTRACT

A compound of the structure

(Abstract continued on next page.)



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl, alkenyl, alkynyl, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl, alkenyl, alkynyl, tri-(alkyl)silyl, tri-(alkyl)silylethyl, triphenylmethyl, benzyl, alkoxymethyl, tri-(alkyl)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl, alkenyl, alkynyl, or phenylalkyl;

X is 5-(2,2-dialkyl)[1,3]dioxanyl, 5-(2,2-dicycloalkyl)[1,3]dioxanyl, 4-(2,2-dialkyl)[1,3]dioxanyl, 4-(2,2-dicycloalkyl)[1,3]dioxanyl, 4-(2,2dialkyl)[1,3-]dioxalanyl, or 4-(2,2-dicycloalkyl)[1,3]dioxalanyl;

b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl group, or a pharmaceutically acceptable salt thereof which is useful as an immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agent.

24 Claims, No Drawings

RAPAMYCIN HYDROXYESTERS

BACKGROUND OF THE INVENTION

This invention relates to hydroxyesters of rapamycin and a method for using them for inducing immunosuppression, and in the treatment of transplantation rejection, graft vs. host disease, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, and hyperproliferative vascular disorders.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in *vitro* and *in vivo* [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31,539 (1978); U.S. Pat. Nos. 3,929,992; and 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

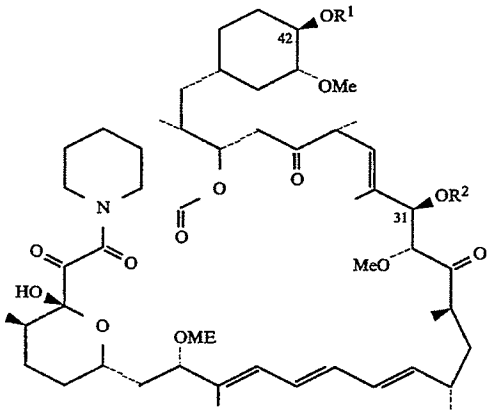
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)], adult T-cell leukemia/lymphoma [European Patent Application 525,960 Al], and ocular inflammation [European Patent Application 532,862 Al].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble aminoacyl prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions.

DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, antiinflammatory, antifungal, antiproliferative, and antitumor agents having the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

b=0–6;

d=0–6; and

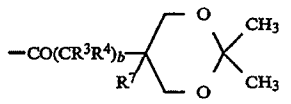
f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

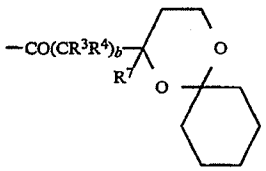
The pharmaceutically acceptable salts are those derived from such inorganic cations such as sodium, potassium, and the like; and organic bases such as: mono-, di-, and trialkyl amines of 1–6 carbon atoms, per alkyl group and mono-. di-, and trihydroxyalkyl amines of 1–6 carbon atoms per alkyl group, and the like.

The terms alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, and alkynyl of 2–7 carbon atoms, include both straight chain as well as branched carbon chains. As the compounds of this invention can contain more than one $-(CR^3R^4)_fOR^{10}$ group, R^3, R^4, f, and R^{10} can be the same or different. Similarly, when other generic substituent descriptions are repeated in the same structure, they can be the same or different.

For a compound in which R^1 contains R^8 and R^9 taken together to form X, where X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, the alkyl group of X contains 1 carbon atom, and d=0, R^1 would have the following structure.



Similarly, for a compound in which R^1 contains R^8 and R^9 taken together to form X, where X is 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, the cycloalkyl group of X contains 6 carbon atom, and d=0, R^1 would have the following structure.



For compounds containing X, preferred compounds include those in which the alkyl group of X, if present, is methyl and the cycloalkyl group of X, if present, is cyclohexyl.

When R^{10} is not hydrogen, alkyl, alkenyl, or alkynyl, it is intended that R^{10} is a group that can serve as an alcohol protecting group. Thus, these groups are intermediates of free hydroxylated compounds, as well as being biologically active in their own right. R^{10} covers tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, and tetrahydropyranyl groups. Other alcohol protecting groups are known by one skilled in the an and are also considered pan of this invention.

Of the compounds of this invention preferred members are those in which R^2 is hydrogen; those in which R^2 is hydrogen, b=0, and d=0; those in which R^2 is hydrogen, b=0, d=0, and R^8 and R^9 are each, independently hydrogen, alkyl, or $-(CR^3R^4)_fOR^{10}$, or are taken together to form X.

Compounds of this invention having the ester group $-CO(CR^3R^4)_bCR^5R^6)_d(CR^7R^8R^9)_e$ at the 42- or 31,42-positions can be prepared by acylation of rapamycin using protected hydroxy and polyhydroxy acids, alkoxy or polyalkoxy carboxylic acids that have been activated, followed by removal of the alcohol protecting groups, if so desired. Several procedures for carboxylate activation are known in the art, but the preferred methods utilize carbodiimides, mixed anhydrides, or acid chlorides. For example, an appropriately substituted carboxylic acid can be activated as a mixed anhydride, with an acylating group such as 2,4,6-trichlorobenzoyl chloride. Treatment of rapamycin with the mixed anhydride under mildly basic condition provides the desired compounds. Alternatively, the acylation reaction can be accomplished with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and dimethylaminopyridine. Mixtures of 42- and 31,42-esters can be separated by chromatography.

The 31-ester-42-hydroxy compounds of this invention can be prepared by protecting the 42-alcohol of rapamycin with a protecting group, such as with a tert-butyl dimethylsilyl group, followed by esterification of the 31-position by the procedures described above. The preparation of rapamycin 42-silyl ethers is described in U.S. Pat. No. B1 5,120,842, which is hereby incorporated by reference. Removal of the protecting group provides the 31-esterified compounds. In the case of the tert-butyl dimethylsilyl protecting group, deprotection can be accomplished under mildly acidic conditions, such as acetic acid/water/THF. The deprotection procedure is described in Example 15 of U.S. Pat. No. 5,118,678, which is hereby incorporated by reference.

Having the 31-position esterified and the 42-position deprotected, the 42-position can be esterified using a different acylating agent than was reacted with the 31-alcohol, to give compounds having different esters at the 31- and 42- positions. Alternatively, the 42-esterified compounds, prepared as described above, can be reacted with a different acylating agent to provide compounds having different esters at the 31-and 42-positions.

This invention also covers analogous hydroxy esters of other rapamycins such as, but not limited to, 29-demethoxyrapamycin, [U.S. Pat. No. 4,375,464, 32-demethoxyrapamycin under C.A. nomenclature]; rapamycin derivatives in which the double bonds in the 1-, 3-, and/or 5-positions have been reduced [U.S. Pat. No. 5,023,262]; 29-desmethylrapamycin [U.S. Pat. No. 5,093,339, 32-desmethylrapamycin under C.A. nomenclature]; 7,29-bisdesmethylrapamycin [U.S. Pat. No. 5,093,338, 7,32-desmethylrapamycin under C.A. nomenclature]; and 15-hydroxyrapamycin [U.S. Pat. No. 5,102,876]. The disclosures in the above cited U.S. Patents are hereby incorporated by reference.

Immunosuppressive activity for representative compounds of this invention was evaluated in an in vitro standard pharmacological test procedure to measure the inhibition of lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The pinch skin graft test procedure measures the immunosuppressive activity of the compound tested as well as the ability of the compound tested to inhibit or treat transplant rejection. The adjuvant arthritis standard pharmacological test procedure, which measures the ability of the compound tested to inhibit immune mediated inflammation. The adjuvant arthritis test procedure is a standard pharmacological test procedure for rheumatoid arthritis. The procedures for these standard pharmacological test procedures are provided below.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative com-

pounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated radioactivity is determined. Inhibition of lymphoproliferation is assessed as percent change in counts per minute from nondrug treated controls. For each compound evaluated, rapamycin was also evaluated for the purpose of comparison. An IC_{50} was obtained for each test compound as well as for rapamycin. When evaluated as a comparator for the representative compounds of this invention, rapamycin had an IC_{50} ranging from 0.6–1.5 nM. The results obtained are provided as an IC_{50} and as the percent inhibition of T-cell proliferation at 0.1 μM. The results obtained for the representative compounds of this invention were also expressed as a ratio compared with rapamycin. A positive ratio indicates immunosuppressive activity. A ratio of greater than 1 indicates that the test compound inhibited thymocyte proliferation to a greater extent than rapamycin. Calculation of the ratio is shown below.

$$\frac{IC_{50} \text{ of Rapamycin}}{IC_{50} \text{ of Test Compound}}$$

Representative compounds of this invention were also evaluated in an in vivo test procedure designed to determine the survival time of pinch skin graft from male BALB/c donors transplanted to male C_3H(H-2K) recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385–402, (1951). Briefly, a pinch skin graft from the donor was grafted on the dorsum of the recipient as a allograft, and an isograft was used as control in the same region. The recipients were treated with either varying concentrations of test compounds intraperitoneally or orally. Rapamycin was used as a test control. Untreated recipients serve as rejection control. The graft was monitored daily and observations were recorded until the graft became dry and formed a blackened scab. This was considered as the rejection day. The mean graft survival time (number of days±S.D.) of the drug treatment group was compared with the control group. The following table shows the results that were obtained. Results are expressed as the mean survival time in days. Untreated (control) pinch skin grafts are usually rejected within 6–7 days. Compounds were tested using a dose of 4 mg/kg.

The adjuvant arthritis standard pharmacological test procedure measures the ability of test compounds to prevent immune mediated inflammation and inhibit or treat rheumatoid arthritis. The following briefly describes the test procedure used. A group of rats (male inbread Wistar Lewis rats) are pre-treated with the compound to be tested (1 h prior to antigen) and then injected with Freud's Complete Adjuvant (FCA) in the right hind paw to induce arthritis. The rats are then orally dosed on a Monday, Wednesday, Friday schedule from day 0–14 for a total of 7 doses. Both hind paws are measured on days 16, 23, and 30. The difference in paw volume (mL) from day 16 to day 0 is determined and a percent change from control is obtained. The left hind paw (uninjected paw) inflammation is caused by T-cell mediated inflammation and is recorded in the above table (% change from control). The right hind paw inflammation, on the other hand, is caused by non-specific inflammation. Compounds were tested at a dose of 5 mg/kg. The results are expressed as the percent change in the uninjected paw at day 16 versus control; the more negative the percent change, the more potent the compound. Rapamycin provided between −70% and −90% change versus control, indicating that rapamycin treated rats had between 70–90% less immune induced inflammation than control rats.

The results obtained in these standard pharmacological test procedures are provided following the procedure for making the specific compounds that were tested.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. The results obtained in the LAF test procedure indicates suppression of T-cell proliferation, thereby demonstrating the immunosuppressive activity of the compounds of this invention. Further demonstration of the utility of the compounds of this invention as immunosuppressive agents was shown by the results obtained in the skin graft and adjuvant arthritis standard pharmacological test procedures. Additionally, the results obtained in the skin graft test procedure further demonstrates the ability of the compounds of this invention to treat or inhibit transplantation rejection. The results obtained in the adjuvant arthritis standard pharmacological test procedure further demonstrate the ability of the compounds of this invention to treat or inhibit rheumatoid arthritis.

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment or inhibition of transplantation rejection such as kidney, heart, liver, lung, bone marrow, pancreas (islet cells), cornea, small bowel, and skin allografts, and heart valve xenografts; in the treatment or inhibition of autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease, pulmonary inflammation (including asthma, chronic obstructive pulmonary disease, emphysema, acute respiratory distress syndrome, bronchitis, and the like), and eye uveitis.

Because of the activity profile obtained, the compounds of this invention also are considered to have antitumor, antifungal activities, and antiproliferative activities. The compounds of this invention therefore also useful in treating solid tumors, adult T-cell leukemia/lymphoma, fungal infections, and hyperproliferative vascular diseases such as restenosis and atherosclerosis. When used for restenosis, it is preferred that the compounds of this invention are used to treat restenosis that occurs following an angioplasty procedure. When used for this purpose, the compounds of this invention can be administered prior to the procedure, during the procedure, subsequent to the procedure, or any combination of the above.

When administered for the treatment or inhibition of the above disease states, the compounds of this invention can be administered to a mammal orally, parenterally, intranasally, intrabronchially, transdermally, topically, intravaginally, or rectally.

It is contemplated that when the compounds of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents.

Such other immunoregulatory agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, rapamycin, cyclosporin A, FK-506, OKT-3, and ATG. By combining the compounds of this invention with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, the lesser amounts of each of the agents are required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and cyclosporin A at subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23: 507 (1991)].

The compounds of this invention can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid. When formulated orally, it has been found that 0.01% Tween 80 in PHOSAL PG-50 (phospholipid concentrate with 1,2-propylene glycol, A. Nattermann & Cie. GmbH) provides an acceptable oral formulation.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions ,and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carders are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily dosages of active compound would be 0.1 μg/kg–100 mg/kg, preferably between 0.001–25 mg/kg, and more preferably between 0.01–5 mg/kg. Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, nasal, or intrabronchial administration will be determined by the administering physician based on experience with the individual subject treated. Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example., packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

The following examples illustrate the preparation and biological activities of representative compounds of this invention.

EXAMPLE 1

Rapamycin 42-ester with (tetrahydropyran-2-yloxy)acetic acid

2,4,6-Trichlorobenzoyl chloride (0.55 mL, 3.51 mmol) was added via syringe to a solution of the glycolic acid THP-ether (0.562 g, 3.51 mmol) and triethylamine (0.49 mL, 3.51 mmol) in 10 mL THF at 0 ° C. under nitrogen. The mixture was stirred for 4 h at room

temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 10 mL benzene, then rapamycin (2.92 g, 3.19 mmol) and DMAP (0.429 g, 3.51 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to an oily yellow solid. Flash chromatography (2X with 65% EtOAc-hexane) afforded the title compound (1.114 g, 33%) as a white solid.

(−)FAB-MS m/z 1055.5 (M−), 590.3 (southern fragment), 463.2 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.60 (m, 1 H, C(42)H), 4.66 (m, 1H), 4.14 (s, 2H), 3.73 (m, 1H), 3.42 (m, 1H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 169.2, 97.4, 63.5, 61.2, 29.7, 24.8. 18.8.

EXAMPLE 2

Rapamycin 42-ester with hydroxyacetic acid

p-Toluenesulfonic acid (10 mg) was added to a solution of the product of Example 1 (306 mg, 0.29 mmol) in 10 mL CH_3OH at 0 ° C. The solution was stirred 2 h at room temperature, then quenched with saturated $NaHCO_3$ solution. The aqueous phase was extracted 3X with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to a white solid. Purification by flash chromatography (2X with EtOAc) afforded the title compound (145 mg, 51%) as a white solid.

(−) FAB-MS m/z 971.3 (M−), 590 (southern fragment), 379.1 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.60 (m, 1H, C(42)H), 3.98 (s, 2H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 172.1, 59.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.80 nM

LAF ratio: 0.83

Percent change in adjuvant arthritis versus control: −88%

EXAMPLE 3

Rapamycin 42-ester with 2.2-dimethyl-3-(tetrahydropyran-2-yloxy)propionic acid

To a solution of the 2,2-dimethyl-3-hydroxypropionic acid THP-ether (0.319 g, 1.58 mmol) and triethylamine (0.22 mL, 1.58 mmol) in 5 mL dry THF at 0 ° C. under nitrogen was added 2,4,6-trichlorobenzoyl chloride (0.25 mL, 1.58 mmol) dropwise via syringe. The mixture was stirred 4.5 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and a warm water bath. The residue was dissolved in 5 mL benzene, then rapamycin (1.31 g, 1.43 mmol) and DMAP (0.193 g, 1.58 mmol) were added. The mixture was stirred overnight at room temperature, diluted with EtOAc, washed with 1N HCl (aq), saturated $NaHCO_3$ (aq), H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow oily solid. Flash chromatography (1X with 60% EtOAc-hexane, 1X with 55% EtOAc-hexane) afforded the title compound (0.356 g, 23%), as a white solid.

(−)FAB-MS m/z 1097.7 (M−), 590.4 (southern fragment), 505.3 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42H), 4.55 (m, 1H), 3.69 (m, 1H), 3.60 (m, 2H), 3.42 (m, 1H), 1.13 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 175.0, 98.0, 73.8, 60.7, 42.6, 30.0, 24.9, 22.0, 21.6, 18.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 7.10 nM

LAF ratio: 0.34

EXAMPLE 4

Rapamycin 42-ester with 3-hydroxy-2,2-dimethylpropionic acid

p-Toluenesulfonic acid (10 mg) was added to a solution of the product of Example 3 (250 mg, 0.23 mmol) in 10 mL CH_3OH at 0 ° C. The solution was stirred 2 hours at room temperature, then quenched with saturated $NaHCO_3$ solution. The aqueous phase was extracted 3X with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to a white solid. Purification by flash chromatography (2X with 75% EtOAc-hexane) afforded the title compound (103 mg, 45%) as a white solid.

(−) FAB-MS m/z 1013.3 (M^{31}), 590.2 (southern fragment), 421.1 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.48 (m, 1H, C(42)H), 3.39 (d, 2H), 106 (s, 6H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 175.5, 68.0, 44.1, 21.7.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}:0.80 nM

LAF ratio: 1.25

Skin graft survival time: 10.7±0.5 days

EXAMPLES 5 AND 6

Rapamycin 42-ester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid (Ex. 5) Rapamycin 31,42-diester with 2,2-dimethyl[1.3]dioxalane-4-carboxylic acid (EX. 6)

2,4,6-Trichlorobenzoyl chloride (0.56 mL, 3.61 mmol) was added via syringe to a solution of the 2,3-dihydroxypropionic acid isopropylidene ketal (0.527 g, 3.61 mmol) and triethylamine (0.50 mL, 3.61 mmol) in 10 mL THF at 0 ° C. under nitrogen. The mixture was stirred 4 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 15 mL benzene and rapamycin (3.00 g, 3.28 mmol), then DMAP (0.441 g, 3.61 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to a yellow foam. Flash chromatography on silica gel (gradient elution: 50–60–75–100% EtOAc-hexane, 4X with 65% EtOAc-hexane) afforded the title compounds. The less polar 31,42-diester (0.415 g) eluted first and the more polar 42-monoester (0.601 g, 16%) eluted second, and were isolated as white solids.

EXAMPLE 5

(−)FAB-MS m/z 1041.4 (M−), 590.3 (southern fragment), 449.2 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.6 (m, 1H, C(42)H), 4.6 (m, 1H), 4.20 (dd, 1H), 3.96 (m, 1H), 1.36 (s, 3H), 1.30 (s, 3H). ^{13}C

NMR (100.6 MHz, d-6 DMSO) δ 170.5, 110.2, 73.4, 66.6, 25.7, 25.4.

EXAMPLE 6

(−)FAB-MS m/z 1169.6 (M−). ^{1}H NMR (400 MHz, d-6 DMSO) δ 5.3 (m, 1H, C(31)H), 4.6 (m, 1H, C(42)H), 4.6 (m, 2H), 4.19 (t, 1H), 4.13 (t, 1H), 3.9 (m, 2H), 1.36 (s, 3H), 1.33 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 170.5, 169.2, 110.3, 110.2, 73.4, 66.6, 66.5, 25.8, 25.7, 25.4, 25.1.

Results obtained in standard pharmacological test procedures:

EXAMPLE 5

LAF IC_{50}: 1.20 nM
LAF ratio: 0.74

EXAMPLE 6

LAF IC_{50}: 1.30 nM
LAF ratio: 0.5

EXAMPLE 7

Rapamycin 42-ester with 2,3-dihydroxypropionic acid

A solution of the product of Example 5 (351 mg, 0.34 mmol) in 10 mL THF and 10 mL 1N HCl was stirred at room temperature for 6 h. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to an oil. Flash chromatography (1X with EtOAc, 1X with 10% MeOH-CH_2Cl_2, 1X with 5% MeOH-EtOAc) afforded the title compound (78 mg, 23%) as a white solid.

(−)FAB-MS m/z 1001.2 (M−), 590.2 (southern fragment), 409.1 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 5 4.5 (m, 1H, C(42)H), 3.60 (m, 1H), 3.45 (m, 2H).

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 1.4 nM
LAF ratio: 0.40

EXAMPLE 8

Rapamycin 42-ester with
2.2-dimethyl[1.3dioxane-5-carboxylic acid

2,4,6-Trichlorobenzoyl chloride (0.98 mL, 6.27 mmol) was added via syringe to a solution of the 2-(hydroxymethyl)-3-hydroxypropionic acid isopropylidene ketal (1.000 g, 6.24 mmol) and triethylamine (0.90 mL, 6.46 mmol) in 20 mL THF at 0 ° C. under nitrogen. The mixture was stirred for 4 h at room temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 20 mL benzene, then rapamycin (5.70 g, 6.24 mmol) and DMAP (0.762 g, 6.24 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. Flash chromatography (75% EtOAc-hexane) afforded the title compound (4.17 g, 63%) as a white solid.

(−)FAB-MS m/z 1055.8 (M−), 590.5 (southern fragment), 463.4 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.95 (m, 4H), 1.30 (s, 6H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 170.1, 97.4, 59.5, 24.8, 22.5.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.76 nM
LAF ratio: 0.45

EXAMPLE 9

Rapamycin 42-ester with
3-hydroxy-2-hydroxymethylpropionic acid

A solution of the product of Example 8 (3.30 g, 3.12 mmol) in 50 mL THF and 25 mL 1N HCl was stirred 2 h at room temperature. The solution was diluted with saturated $NaHCO_3$ solution and extracted with EtOAc (3X). The combined orgamic phases were washed with saturated NaCl (aq), dried over $MgSO_4$, filtered and concentrated to a yellow foam. Purification by flash chromatography (1X with EtOAc; 2X with 5% EtOH-EtOAc) afforded the title compound (1.68 g, 53 %) as a white solid.

(−)FAB-MS m/z 1015.5 (M−), 590.3 (southern fragment), 423.3 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.6 (br s, 2H), 4.55 (m, 1H, C(42)H), 3.55 (m, 4H), 2.57–2.53 (m, 1H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 172.2, 59.3, 51.5.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.84 nM
LAF ratio: 0.57

EXAMPLE 10

Rapamycin 42-ester with
2,2,5-trimethyl[1.3dioxane-5-carboxylic acid

To a solution of the 2,2-bis(hydroxymethyl)propionic acid isopropylidene ketal (1.041 g, 5.98 mmol) (prepared according to the procedure of Bruice, J. Am. Chem. Soc. 89:3568 (1967)) and triethylamine (0.83 mL, 5.98 mmol) in 20 mL anhydrous THF at 0 ° C. under nitrogen was added 2,4,6-trichlorobenzoyl chloride (0.93 mL, 5.98 mmol) and the resultant white suspension was stirred 5 h at room temperature. The precipitate was removed by vacuum filtration, rinsing the flask and filter cake with an additional 10 mL dry THF. The filtrate was concentrated by rotary evaporation to a white solid. The residue was dissolved in 20 mL dry benzene, then rapamycin (5.47 g, 5.98 mmol) and DMAP (0.731 g, 5.98 retool) were added. After stirring overnight at room temperature, the mixture was diluted with EtOAc, washed with H_2O and saturated NaCl (aq), dried over $MgSO_4$, filtered and evaporated to a yellow oil. Flash chromatography (5X with 60% EtOAc-hexane) afforded the title compound (2.2 g, 34%) as a white solid.

(−)FAB-MS m/z 1069.5 (M−), 590.3 (southern fragment), 477.2 (northern fragment). ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.57 (m, 1H, C(42)H, 4.02 (d, 2H), 3.60 (d, 2H), 1.34 (s, 3H), 1.24 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.2, 99.0, 65.0, 22.2, 18.1.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 4.90 nM
LAF ratio: 0.41
Skin graft survival time: 11.0±1.3 days

EXAMPLE 11

Rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid

A solution of the product of Example 10 (2.8 g, 2.65 mmol) in 50 mL THF and 25 mL 1N HCl was stirred at room temperature for 4 h. The mixture was diluted with water and extracted three times with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution, saturated NaCl solution, dried over $MgSO_4$, filtered and evaporated to a yellow oily solid. Purification by flash chromatography (3X with EtOAc) afforded the title compound (1.6 g, 59%).

(−)FAB-MS m/z 1029.6 (M^-), 590.4 (southern fragment), 437.3 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.5 (m, 1H, C(42)H), 3.45 (s, 4H), 1.04 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 174.2, 63.7, 63.6, 49.9, 16.8.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 0.80 and 1.80 nM

LAF ratio: 1.00 and 0.44

Skin graft survival time: 11.4±1.5 and 12.0±1.1 days

Percent change in adjuvant arthritis versus control: −88%

EXAMPLE 12

Rapamycin 42-ester with 2,2-dimethyl-5-(2-trimethylsilanylethoxymethyl)[1,3]dioxane-5-carboxylic acid

2,4,6-Trichlorobenzoyl chloride (0.14 mL, 0.86 mmol) was added via syringe to a solution of the 2,2-bis(hydroxymethyl)-2-(2-trimethylsilylethoxy)propionic acid isopropylidene ketal (0.250 g, 0.86 mmol) and triethylamine (0.12 mL, 0.86 mmol) in 2 mL THF at 0 ° C. under nitrogen. The mixture was stirred for 4 h at room temperature, and a white precipitate formed. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 2 mL benzene, then rapamycin (0.786 g, 0.86 mmol) and DMAP (0.105 g, 0.86 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. Flash chromatography (gradient elution: 40–60–80–100% EtOAc-hexane) afforded the title compound (0.559 g, 54%) as a white solid.

(−)FAB-MS m/z 1185.2 (M^-), 590.1 (southern fragment), 593 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.73 (m, 4H), 3.57 (s, 2 H), 3.43 (t, 2H), 1.29 (s, 6H), 0.79 (t, 2H), −0.04 (s, 9H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 171.1, 97.7, 70.2, 68.1, 61.3, 46.0, 24.6, 22.1, 14.6, −1.3.

Results obtained in standard pharmacological test procedures:

LAF IC_{50}: 7.20 nM

LAF ratio: 0.05

EXAMPLES 13 and 14

Rapamycin 42-ester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid (Ex. 13)

Rapamycin 31.42-diester with 3-methyl-1.5-dioxa-spiro[5.5]undecane 3-carboxylic acid (Ex. 14)

2,4,6-Trichlorobenzoyl chloride (0.16 mL, 1.0 mmol) was added via syringe to a solution of the 2,3-dihydroxypropionic acid cyclohexylidene ketal (0.214 g, 1.0 mmol) and triethylamine (0.14 mL, 1.0 mmol) in 2.5 mL THF at 0 ° C. under nitrogen. The mixture was stirred 4 h at room temperature. The white precipitate was removed by vacuum filtration and the filtrate was concentrated with a stream of nitrogen and warm water bath. The residue was dissolved in 3 mL benzene and rapamycin (0.457 g, 0.5 mmol), then DMAP (0.061 g, 0.5 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc, washed with cold 1N HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated to a yellow foam. Flash chromatography on silica gel (45–50% EtOAc-hexane) afforded the title compounds. The 31,42-diester (0.168 g, 26%) eluted first and the more polar 42-monoester (0.301 g, 52%) eluted second, and the products were isolated as white solids.

EXAMPLE 13

(−)FAB-MS m/z 1109.5 (M^-), 590.3 (southern fragment), 517.3 (northern fragment). ^1H NMR (400 MHz, d-6 DMSO) δ 4.55 (m, 1H, C(42)H), 3.61 (t, 4H), 1.04 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.3, 97.2, 64.2.

EXAMPLE 14

(−)FAB-MS m/z 1305.6 (M^-). ^1H NMR (400 MHz, d-6 DMSO) δ 5.25 (m, 1H, C(31)H), 4.55 (m, 1H, C(42)H), 3.64–3.54 (m, 8H), 1.05 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (100.6 MHz, d-6 DMSO) δ 173.2, 172.1, 97.3, 97.2, 64.3, 64.2, 63.9.

Results obtained in standard pharmacological test procedures:

EXAMPLE 13

LAF IC_{50}: 0.6 nM

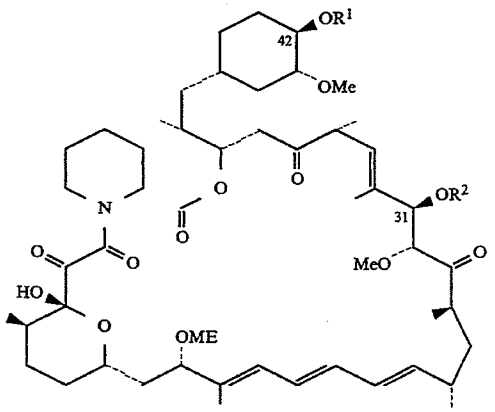
LAF ratio: 2.00

EXAMPLE 14

LAF: inhibited T-cell proliferation by 43% at 0.1 μM

What is claimed is:

1. A compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms,

alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$—F, or$—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

b=0–6;

d=0–6 ; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

2. The compound of claim **1**, wherein R^2 is hydrogen or a pharmaceutically acceptable salt thereof.

3. The compound of claim **2**, wherein b=0 and d=0 or a pharmaceutically acceptable salt thereof.

4. The compound of claim **3**, wherein R^8 and R^9 are each, independently hydrogen, alkyl, or $—(CR^3R^4)_fOR^{10}$, or are taken together to form X or a pharmaceutically acceptable salt thereof.

5. The compound of claim **1** which is rapamycin 42-ester with (tetrahydropyran-2-yloxy)acetic acid or a pharmaceutically acceptable salt thereof.

6. The compound of claim **1** which is rapamycin 42-ester with hydroxyacetic acid or a pharmaceutically acceptable salt thereof.

7. The compound of claim **1** which is rapamycin 42-ester with 2,2-dimethyl-3-(tetrahydropyran-2-yloxy)propionic acid or a pharmaceutically acceptable salt thereof.

8. The compound of claim **1** which is rapamycin 42-ester with 3-hydroxy-2,2-dimethylpropionic acid or a pharmaceutically acceptable salt thereof.

9. The compound of claim **1** which is rapamycin 42-ester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid or a pharmaceutically acceptable salt thereof.

10. The compound of claim **1** which is rapamycin 31,42-diester with 2,2-dimethyl[1,3]dioxalane-4-carboxylic acid or a pharmaceutically acceptable salt thereof.

11. The compound of claim **1** which is rapamycin 42-ester with 2,3-dihydroxypropionic acid or a pharmaceutically acceptable salt thereof.

12. The compound of claim **1** which is rapamycin 42-ester with 2,2-dimethyl[1,3]dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

13. The compound of claim **1** which is rapamycin 42-ester with 3-hydroxy-2-hydroxymethylpropionic acid or a pharmaceutically acceptable salt thereof.

14. The compound of claim **1** which is rapamycin 42-ester with 2,2,5-trimethyl[1,3]dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

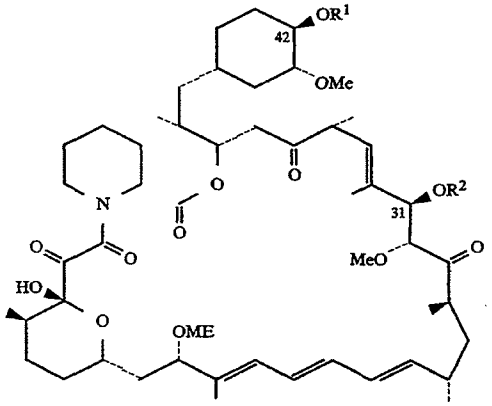
15. The compound of claim **1** which is rapamycin 42-ester with 2,2-bis(hydroxymethyl)propionic acid or a pharmaceutically acceptable salt thereof.

16. The compound of claim **1** which is rapamycin 42-ester with 2,2-dimethyl-5-(2-trimethylsilanylethoxymethyl)[1,3]-dioxane-5-carboxylic acid or a pharmaceutically acceptable salt thereof.

17. The compound of claim **1** which is rapamycin 42-ester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid or a pharmaceutically acceptable salt thereof.

18. The compound of claim **1** which is rapamycin 31,42-diester with 3-methyl-1,5-dioxa-spiro[5.5]undecane 3-carboxylic acid or a pharmaceutically acceptable salt thereof.

19. A method of treating transplantation rejection or graft vs. host disease in a mammal in need thereof, which comprises administering to said mammal an antirejection effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of

3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with —$(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —$(CR^3R^4)_fOR^{10}$, —CF_3, —F, or —CO_2R^{11};

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —$(CR^3R^4)_fOR^{10}$, —CF_3, —F, or —CO_2R^{11}, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with —$(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

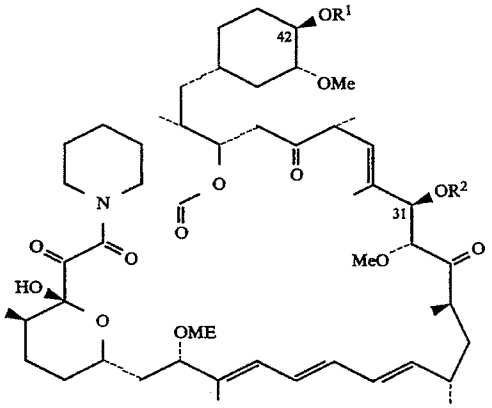
b=0–6;

d=0–6 ; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one —$(CR^3R^4)_fOR^{10}$, X, or —$(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

20. A method of treating a fungal infection in a mammal in need thereof, which comprises administering to said mammal an antifungal effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or —$CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —$(CR^3R^4)_fOR^{10}$, —CF_3, —F, or —CO_2R^{11}, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with —$(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —$(CR^3R^4)_fOR^{10}$, —CF_3, —F, or —CO_2R_{11};

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, —$(CR^3R^4)_fOR^{10}$, —CF_3, —F, or —CO_2R^{11}, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with —$(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

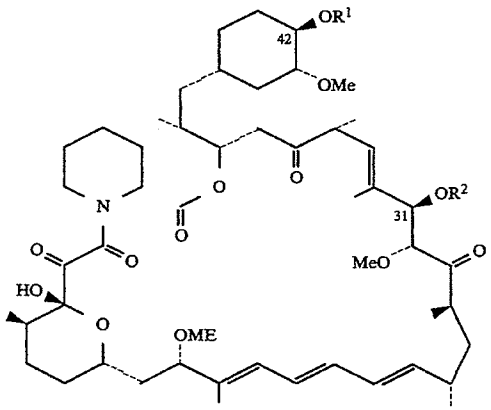
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one —$(CR^3R^4)_fOR^{10}$, X, or —$(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

21. A method of treating rheumatoid arthritis in a mammal in need thereof, which comprises administering to said mammal an antiarthritis effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or —$CO(CR^3R^4)_b(CR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms,

alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

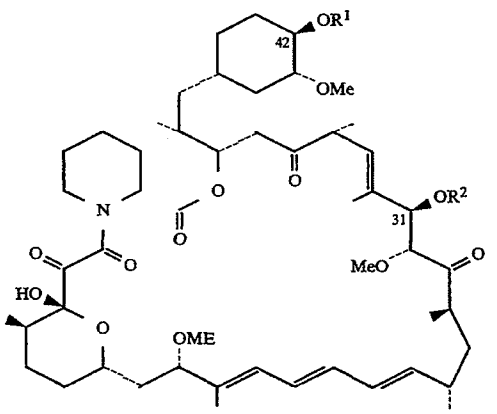
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

22. A method of treating restenosis in a mammal in need thereof, which comprises administering to said mammal an antiproliferative effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_bCR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

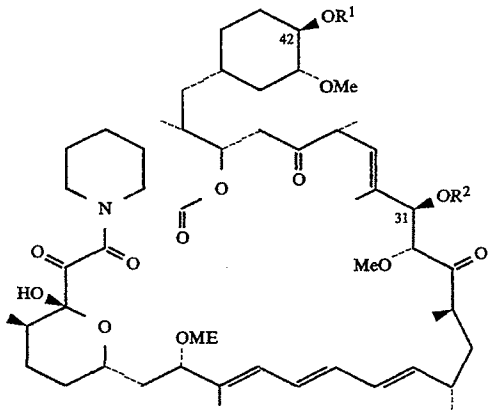
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

23. A method of treating pulmonary inflammation in a mammal in need thereof, which comprises administering to said mammal an antiinflammatory effective amount of a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_bCR^5R^6)_dCR^7R^8R^9$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

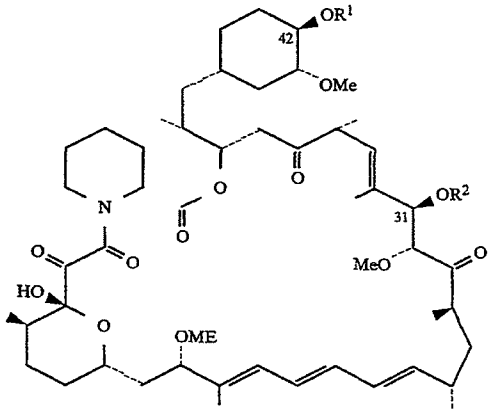
b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof.

24. A pharmaceutical composition which comprises a compound of the structure



wherein R^1 and R^2 are each, independently, hydrogen or $—CO(CR^3R^4)_bCR^5R^6)_dCR^7R^8R^9$; R^3 and R^4 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, trifluoromethyl, or —F;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$, or R^5 and R^6 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^7 is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$, —F, or $—CO_2R^{11}$;

R^8 and R^9 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, $—(CR^3R^4)_fOR^{10}$, $—CF_3$—F, or $—CO_2R^{11}$, or R^8 and R^9 may be taken together to form X or a cycloalkyl ring of 3–8 carbon atoms that is optionally mono-, di-, or tri-substituted with $—(CR^3R^4)_fOR^{10}$;

R^{10} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silyl, tri-(alkyl of 1–6 carbon atoms)silylethyl, triphenylmethyl, benzyl, alkoxymethyl of 2–7 carbon atoms, tri-(alkyl of 1–6 carbon atoms)silylethoxymethyl, chloroethyl, or tetrahydropyranyl;

R^{11} is hydrogen, alkyl of 1–6 carbon atoms, alkenyl of 2–7 carbon atoms, alkynyl of 2–7 carbon atoms, or phenylalkyl of 7–10 carbon atoms;

X is 5-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 5-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxanyl, 4-(2,2-di-(alkyl of 1–6 carbon atoms))[1,3]dioxalanyl, or 4-(2,2-di-(cycloalkyl of 3–8 carbon atoms))[1,3]dioxalanyl;

b=0–6;

d=0–6; and

f=0–6

with the proviso that R^1 and R^2 are both not hydrogen and further provided that either R^1 or R^2 contains at least one $—(CR^3R^4)_fOR^{10}$, X, or $—(CR^3R^4)_fOR^{10}$ substituted cycloalkyl of 3–8 carbon atoms group, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,362,718

DATED : November 8, 1994

INVENTORS : Jerauld S. Skotnicki, Christina L. Leone, Guy A. Schiehser

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On Page 2 of the Abstract, first column, and in the Patent column 2, lines 1-19; column 14, lines 45-64; column 16, lines 38-57; column 17, lines 41-60; column 18, lines 45-64; column 19, lines 48-67; column 21, lines 1-19; and column 22, lines 3-22, please delete the structure and insert therefor:

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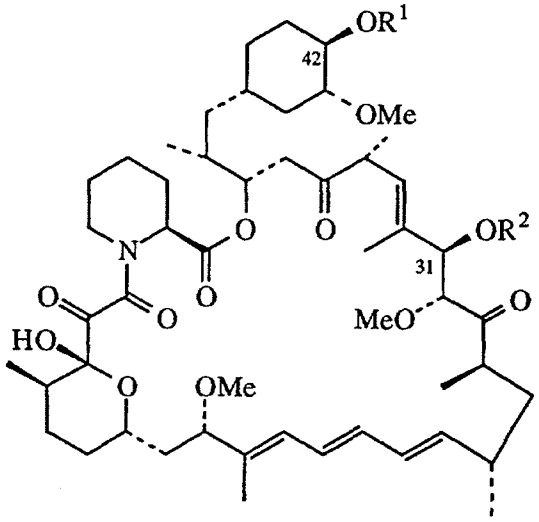

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Signed and Sealed this

Twelfth Day of August, 1997

Bruce Lehman

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

US005516781A

United States Patent [19]

Morris et al.

[11] Patent Number: 5,516,781

[45] Date of Patent: *May 14, 1996

[54] **METHOD OF TREATING RESTENOSIS WITH RAPAMYCIN**

[75] Inventors: **Randall E. Morris**, Los Altos; **Clare R. Gregory**, Menlo Park, both of Calif.

[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,288,711.

[21] Appl. No.: **238,305**

[22] Filed: **May 12, 1994**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 980,000, Nov. 23, 1992, abandoned, which is a continuation of Ser. No. 819,314, Jan. 9, 1992, abandoned.

[51] **Int. Cl.**[6] **A61K 31/345**

[52] **U.S. Cl.** **514/291**

[58] **Field of Search** 514/291, 56; 424/122

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Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

5 Claims, No Drawings

METHOD OF TREATING RESTENOSIS WITH RAPAMYCIN

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. patent application Ser. No. 07/980,000, filed Nov. 23, 1992, now abandoned, which is a continuation of U.S. patent application Ser. No. 07/819,314, filed Jan. 9, 1992, now abandoned.

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial lining. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Intimal thickening following arterial injury can be divided into three sequential steps: 1) initiation of smooth muscle cell proliferation following vascular injury, 2) smooth muscle cell migration to the intima, and 3) further proliferation of smooth muscle cells in the intima with deposition of matrix. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., *Lab. Invest,* 41:407 (1979); Clowes, A., *Circ. Res.* 56:139 (1985); Clowes, A., J, *Cardiovas. Pharm.* 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9:289 (1989); Forrester, J., *J. Am. Coil. Cardiol.* 17:758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Artherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenisis of artherosclerosis. Artherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P. F., *Artherosclerosis Lab. Invest.* 55:5 (1986)].

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others

have shown that deendothelilization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S. M., *Human Pathology* 18:240 (1987); Fingerle, J., *Ateriosclerosis* 10:1082 (1990)]. Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R. G., *JAMA* 265:2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30–40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., *Circulation* 81:1753 (1990); Hardoff, R., *J. Am. Coll. Cardiol.* 15 1486 (1990)].

In an attempt to prevent restenosis or reduce intimal smooth muscle cell proliferation following angioplasty, numerous pharmaceutical agents have been employed clinically, concurrent with or following angioplasty. Most pharmaceutical agents employed in an attempt to prevent or reduce the extent of restenosis have been unsuccessful. The following list identifies several of the agents for which favorable clinical results have been reported: lovastatin [Sahni, R., *Circulation* 80 (Suppl.) 65 (1989); Gellman, J., *J. Am. Coll. Cardiol.* 17:251 (1991)]; thromboxane A_2 synthetase inhibitors such as DP-1904 [Yabe, Y., *Circulation* 80 (Suppl.) 260 (1989)]; eicosapentanoic acid [Nye, E., *Aust. N.Z. J. Med.* 20:549 (1990)]; ciprostene (a prostacyclin analog) [Demke, D., *Brit. J. Haematol* 76 (Suppl.): 20 (1990); Darius, H., *Eur. Heart J.* 12 (Suppl.): 26 (1991)]; trapidil (a platelet derived growth factor) [Okamoto, S., *Circulation* 82 (Suppl.): 428 (1990)]; angiotensin convening enzyme inhibitors [Gottlieb, N., *J. Am. Coll. Cardiol.* 17 (Suppl. A): 181A (1991)]; and low molecular weight heparin [de Vries, C., *Eur. Heart J.* 12 (Suppl.): 386 (1991)].

In an attempt to develop better agents for preventing or reducing smooth muscle proliferation and intimal thickening, the use of balloon catheter induced arterial injury in a variety of mammals has been developed as a standard model of vascular injury that will lead to intimal thickening and eventual vascular narrowing. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab, Invest,* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. Many compounds have been evaluated in this standard animal model. The immunosuppressive agent cyclosporin A has been evaluated and has produced conflicting results. Jonasson reported that cyclosporin A caused an inhibition of the intimal proliferative lesion following arterial balloon catheterization in vivo, but did not inhibit smooth muscle cell proliferation in vitro. [Jonasson, L., *Proc. Natl. Acad. Sci.* 85:2303 (1988)]. Ferns, however reported that when de-endothelilized rabbits were treated with cyclosporin A, no significant reduction of intimal proliferation was observed in vivo. Additionally, intimal accumulations of foamy macrophages, together with a number of vacuolated smooth muscle cells in the region adjacent to the internal elastic lamina were observed, indicating that cyclosporin A may modify and enhance lesions that form at the sites of arterial injury. [Ferns, G. A., *Circulation* 80 (Supp): 184 (1989); Ferns, G., *Am. J. Path.* 137:403 (1990)].

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* [U.S. Pat. No. 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., *Can. J. Physiol. Pharm.* 55:48 (1977)], inhibit murine T-cell activation [Staruch, M., *FASEB* 3:3411 (1989)], prolong survival time of organ gratis in histoincompatible rodents [Morris, R., *Med. Sci. Res.* 17:877 (1989)], and inhibit transplantation rejection in mammals [Calne, R., European Patent Application 401,747]. Rapamycin blocks calcium-dependent, calcium-independent, cytokine-independent and constitutive T and B cell division at the G1-S interface. Rapamycin inhibits gamma-interferon production induced by Il -1 and also inhibits the gamma-interferon induced expression of membrane antigen. [Morris, R. E., *Transplantation Rev.* 6:39 (1992)]. The use of rapamycin in preventing coronary graft atherosclerosis (CGA) in rats has been disclosed by Meiser [*J. Heart Lung Transplant* 9:55 (1990)]. Arterial thickening following transplantation, known as CGA, is a limiting factor in graft survival that is caused by a chronic immunological response to the transplanted blood vessels by the transplant recipient's immune system. [Dec. G, *Transplantation Proc.* 23:2095 (1991) and Dunn, M. *Lancet* 339:1566 (1992)]. The disclosed invention is distinct from the use of rapamycin for preventing CGA, in that CGA does not involve injury to the recipients own blood vessels; it is a rejection type response. The disclosed invention is related to vascular injury to native blood vessels. The resulting intimal smooth muscle cell proliferation dose not involve the immune system, but is growth factor mediated. For example, arterial intimal thickening after balloon catheter injury is believed to be caused by growth factor (PGDF, bFGF, TGFb, IL-1 and others)-induced smooth muscle cell proliferation and migration. [Ip, J. H., *J. Am. Coll. Cardiol* 15:1667 (1990)]. Ferns has also shown that the immune response is not involved in arterial intimal thickening following balloon catheterization, as he found that there was no difference in intimal thickening between arteries from athymic nude rats (rats lacking T-cells) and normal rats after balloon catheterization [*Am. J. Pathol.* 138:1045 (1991)].

DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium. Rapamycin is also useful in preventing intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion resulting from mechanically mediated injury. In particular, for the prevention of restenosis following a percutaneous transluminal coronary angioplasty procedure.

Treating includes retarding the progression, arresting the development, as well as palliation. Preventing includes inhibiting the development of and prophylacticly preventing of hyperproliferative vascular disease in a susceptible mammal.

This invention also provides a method of using a combination of rapamycin and mycophenolic acid for the same utilities described above. Mycophenolic acid, an antiproliferative antimetabolite, inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, enzymes in the de novo purine biosynthetic pathway. This results in an inhibition of DNA synthesis which causes an accumulation of cells at the G 1-S interface. Other combinations containing rapamycin that are useful for preventing or treating hyperproliferative vascular disease will be apparent to one skilled in the art. These include, but are not limited to, using rapamycin in combination with other antiproliferative antimetabolites.

The effect of rapamycin on hyperproliferative vascular disease was established in an in vitro and an in vivo standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. Cycloporin A was also evaluated in these test procedures for the purpose of comparison. The combination of rapamycin and mycophenolic acid was evaluated in the in vivo test procedure. The procedures and the results obtained are described below.

Rapamycin and cyclosporin A were evaluated in an in vitro standard pharmacological test procedure which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Results were obtained by measuring DNA and protein synthesis in rat smooth muscle cells that have been stimulated with a growth factor such as fetal calf serum or a hypertrophic mitogen, such as angiotensin II. The following briefly describes the procedure that was used. Rat smooth muscle cells were maintained in a 1:1 mixture of defined Eagle's medium (DEM) and Ham's F12 medium with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL) and 25 mL Hepes at pH 7.4. Cells were incubated at 37° C. in a humidified atmosphere of 5% CO_2 with media changes every 2–3 days. Each compound tested was diluted with an appropriate vehicle to obtain a 1 mM stock solution. Ethanol was used as the vehicle for rapamycin and 20% tween 80 in ethanol was the vehicle for cyclosporin A. Test concentrations of drug were obtained by diluting appropriate concentrations of stock solution with serum free media. The smooth muscle cell culture was maintained in a defined serum free media containing 1:1 DEM and Ham's F12 medium, insulin (5×10^{-7}M), transferrin (5 μg/mL), and ascorbate (0.2 mM) for 72 hours before testing in a multi-well plate. After the 72 hour period, an appropriate quantity of stock solution containing either rapamycin or cyclosporin A was added to the smooth muscle cell culture and media mixture. After a 24 hours the appropriate growth factor was added. For the measurement of DNA synthesis, ^{3}H-thymidine was added at 12 hours after the growth factor was added, and the cells were harvested at 36 hours. For the measurement of protein synthesis, ^{3}H-leucine was added at 14 hours after the growth factor was added, and the cells were harvested at 18 hours. The amount of incorporated radioactive label was measured on a scintillation counter.

The following table shows the results obtained for rapamycin on DNA and protein synthesis in smooth muscle cells that were stimulated with 10% fetal calf serum, as measured by incorporation of tritiated thymidine or leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only was normalized to 100%, and the results for cells treated with fetal calf serum or fetal calf serum plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON DNA AND PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH FETAL CALF SERUM*

	^{3}H-Thymidine Incorporation (% of Media)	^{3}H-Leucine Incorporation (% of Media)
Media	100%	100%
FCS	495%	174%
1000 nM RAP + FCS	136%	95%
100 nM RAP + FCS	172%	91%
10 nM RAP + FCS	204%	74%
1 nM RAP + FCS	403%	106%

*Abbreviations:
RAP = rapamycin;
Media = defined serum free media; and
FCS = 10% fetal calf serum.

The following table shows the results obtained for rapamycin on protein synthesis in smooth muscle cells that were stimulated with 10^{-6} nM angiotensin II, as measured by incorporation of tritiated leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only were normalized to 100%, and the results for cells treated with angiotensin or angiotensin plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH ANGIOTENSIN II*

	^{3}H-Leucine Incorporation (% of Media)
Media	100%
ANG	159%
1000 nM RAP + ANG	53%
100 nM RAP + ANG	57%
10 nM RAP + ANG	61%
1 nM RAP + ANG	60%

*Abbreviations:
RAP = rapamycin;
Media = defined serum free media; and
ANG = 10^{-6} nM angiotensin II.

The results of the standard in vitro test procedure showed that rapamycin had a pronounced antiproliferative effect in the presence of FCS and an anti-hypertrophic effect in the presence of angiotensin II. Following vascular injury, DNA and protein synthesis of smooth muscle cells are necessary for the development of restenosis to occur. These results showed that rapamycin inhibited both DNA and protein synthesis in stimulated smooth muscle cells. An antiproliferative effect was also observed with cyclosporin A; however, at 1000 nM, cyclosporin A was cytotoxic and not merely cytostatic. At 1000 nM, cyclosporin A caused lysis of the smooth muscle cells as evidenced by the presence of lactic acid dehydrogenase in the supernatant of the cell culture. Similar toxicity to smooth muscle cells was not observed for rapamycin.

Rapamycin, rapamycin plus mycophenolic acid, and cyclosporin A were evaluated in an in vivo standard pharmacological test procedure that emulates the vascular injury suffered and restenosis that develops following percutaneous transluminal coronary angioplasty in humans. The ability of a test compound to inhibit restenosis was determined by comparing intimal thickening in mammals treated with test compound following balloon catheterization versus intimal thickening in untreated control mammals after the same test procedure. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest,* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab, Invest,* 49:208 (1983); Clowes, A. W., J. *Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. The following briefly describes the procedure that was used. The left carotid arteries of male Sprague-Dawley rats were injured with an inflated 2 Fr balloon catheter. During a 14 day postoperative period, these rats were divided into groups and treated daily with rapamycin (1.5 mg/kg; i.p.), rapamycin plus mycophenolic acid (1.5 mg/kg; i.p.+ 40 mg/kg; p.o.), or cyclosporin A (3 mg/kg; i.p.). Treatment was administered on days 0 to 13 postoperatively. Additionally, one group each also received rapamycin (6 mg/kg/day; i.p.) or cyclosporin A (40 mg/kg/day; i.p.) for two days postoperatively, and then received no treatment for the next 12 days. An untreated group was used an injured control to establish the amount of intimal growth in the absence of treatment. The right carotid was used as an uninjured control in all groups. After the 14-day period, the rats were sacrificed, the carotids removed. The mean areas of the intima and blood vessel wall were measured by morphometry. Results are expressed as an intima percent which can be expressed according to the following formula:

$$\frac{\text{area of intima}}{\text{area of vessel}} * 100$$

The following table shows the results that were obtained.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)*

Test Group	Intima Percent ± S.E.
Uninjured Control	0.00 ± 0.00
Untreated Injured Control	33.3 ± 19.66
RAP (1.5 mg/kg - 14 days)	6.78 ± 4.69
RAP (6 mg/kg - 2 days)	16.56 ± 6.22
RAP + MPA (14 days)	1.6 ± 3.5
CsA (3 mg/kg - 14 days)	26.46 ± 27.42
CsA (40 mg/kg - 2 days)	31.14 ± 20.66

*Abbreviations:
RAP = rapamycin;
MPA = mycophenolic acid; and
CsA = cyclosporin A.

These results show that treatment with rapamycin (1.5 mg/kg for 14 days) resulted in an 80% decrease in the mean percentage intimal thickening compared with the untreated injured control group. Similarly, treatment with the combination of rapamycin and mycophenolic acid produced almost a complete inhibition of intimal thickening (95% reduction in intimal thickening compared with untreated injured control). Cyclosporin A failed to produce any meaningful reduction in intimal thickening.

Similar results were obtained when rapamycin was evaluated at different doses in the above in vivo standard phar-

macological test procedure that emulates the vascular injury that occurs following a percutaneous transluminal coronary angioplasty procedure in humans. Rapamycin was administered on postoperative days 0–13, and examination by morphometry was performed on day 14. Rapamycin, at a dose of 1.5 and 3 mg/kg significantly arrested the development of restenosis as measured by the intima percent 14 days after balloon catheterization, whereas restenosis was clearly observed in the untreated injured control group. These results are summarized in the table below.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	6 mg/kg	0–13	30.92 ± 4.06
Rapamycin	3 mg/kg	0–13	22.68 ± 6.28
Rapamycin	1.5 mg/kg	0–13	21.89 ± 4.2

The results of the in vitro and in vivo standard test procedures demonstrate that rapamycin and rapamycin in combination with mycophenolic acid are useful in treating hyperproliferative vascular disease.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Rapamycin and rapamycin plus mycophenolic acid were also evaluated in a modification of the in vivo test procedure described above. In the modified test procedure, treatment with rapamycin or rapamycin plus mycophenolic acid were stopped on day 14, as above, but the animals were not sacrificed immediately. Intimal thickening was observed when the animals were sacrificed 1, 2, 4 weeks, and 44 days alter treatment had been stopped. Microscopic analysis showed that endothelium regeneration had not occurred during the two week treatment period. For example, 44 days after undergoing balloon catheterization procedure of the carotid artery, untreated injured control rats had an intima percent (±S.E.) of 62.85±3.63, and rats treated with rapamycin+mycophenolic acid (1.5/40 mg/kg) on postoperative days 0–13 had an intima percent (±S.E.) of 50.39±2.58. Better results were not obtained when the same regimen was administered on days 0–30 (intima percent (±S.E.) of 53.55± 2.85). Following cessation of treatment with rapamycin or rapamycin plus mycophenolic acid intimal proliferation, that was previously suppressed, was able to occur. These results are consistent with the results shown in the table above, in which treatment for 2 days with rapamycin followed by 12 days of no treatment inhibited intimal thickening to a lesser degree than treatment with rapamycin for 14 days. These results are expected, as in the absence on an integral endothelial layer, the intimal smooth muscle cells will proliferate. It has been shown that intimal smooth muscle cell growth does not have an inhibitory effect on normal endothelial regeneration, and that intimal smooth muscle cell proliferation ceases when the endothelial layer is established. [Reidy, M., *Lab. Invest.* 59:36 (1988); Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979)]. As such, treatment with rapamycin or rapamycin in combination with mycophenolic acid should be employed so long as the beneficial effect is seen. As the degree of restenosis can be monitored by angiographic and sonographic techniques, the dosage necessary to sustain the opened vessels can be adjusted.

To evaluate the ability of rapamycin and rapamycin plus mycophenolic acid to prevent restenosis following an angioplasty procedure, rapamycin was evaluated in the same in vivo standard pharmacological test procedure for restenosis that was described above, except that treatment with rapamycin began three days before (day-3) the angioplasty procedure was performed. The following table shows the results obtained on day 14 following balloon catheterization of the carotid artery on day 0. Results for treatment from day 3 to 13 are also provided.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	1.5 mg/kg	–3–13*	9.85 ± 1.15
Rapamycin	1.5 mg/kg	–3–3	30.7 ± 6.67
Rapamycin	1.5 mg/kg	–3–0	37.31 ± 4.33
Rapamycin	1.5 mg/kg	3–13	44.38 ± 5.49

*Treatment from three days pre-balloon catheterization to day 13 days post-catheterization.

The results in the table above show that rapamycin prevented the development of restenosis following a balloon angioplasty procedure of the carotid artery, when rapamycin was administered from three days pre-angioplasty until day 13. Treatment from day minus 3 until day 3 or day 0 afforded a lesser degree of prevention, and treatment from day 3 to day 13 did not prevent restenosis.

The effect of rapamycin plus mycophenolic acid (MPA) was also evaluated in the angioplasty standard pharmacological test procedure. The table below shows the results obtained where rats underwent a balloon catheterization procedure of the carotid artery on day 0, and were sacrificed and examined morphometrically on day 44. The treatment regimen is described in the table.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			62.85 ± 3.63
Rapamycin + MPA	40/1.5 mg/kg	0–13	50.39 ± 2.58

-continued

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Rapamycin + MPA	40/1.5 mg/kg	0–30	53.55 ± 2.85
Rapamycin + MPA	40/1.5 mg/kg	–3–13	18.76 ± 10.6

These results show that treatment with rapamycin and mycophenolic acid from day minus 3 to day 13 did effectively prevent restenosis at day 44, whereas the regimens which did not include drug administration before the angioplasty procedure did not effectively prevent restenosis at day 44.

Similar results were obtained when rat thoracic aortas were subjected to a balloon catheterization procedure, as described above, on day 0. The rats were either sacrificed and examined on day 14 or on day 44. The results obtained with rapamycin and rapamycin plus mycophenolic acid (MPA) are shown in the table below.

EFFECT OF RAPAMYCIN AND RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED THORACIC AORTAS

Group	Dose	Treatment Days	Intima Percent ± S.E.
	Day 14 results		
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			15.52 ± 2.99
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00
	Day 44 Results		
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			28.76 ± 6.52
Rapamycin	1.5 mg/kg	–3–13	0.00 ± 0.00
Rapamycin + MPA	40/1.5 mg/kg	–3–13	8.76 ± 3.34

The results in the table above show that treatment with rapamycin from 3 days preoperatively until 13 days postoperatively completely prevented the development of restenosis 44 days after a balloon catheterization of the thoracic aorta. Using the same treatment regimen, rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterization and significantly prevented restenosis 44 days following balloon catheterization.

Similarly, day minus 3 to day 13 treatment with rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterizaton of the abdominal aortas in rats. These results are shown in the table below.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED ABDOMINAL AORTAS (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			10.17 ± 2.42
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00

The results in the tables above show that rapamycin, alone or in combination with mycophenolic acid, is useful in preventing restenosis following invasive procedures that disrupt the vascular endothelial lining, such as percutaneous transluminal coronary angioplasty, vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures. These data also show that the administration of rapamycin, alone or in combination with mycophenolic acid, from 3 days pre-catheterization to 13 days post-catheterization, allowed the endothelium to heal, while preventing intimal smooth muscle cell proliferation. That intimal proliferation did not occur 31 days after administration with rapamycin, alone or in combination with mycophenolic acid, had been stopped, demonstrates that the endothelial layer had regenerated, as intimal proliferation stops after the reestablishment of the endothelial layer. The reestablishment of an intact endothelial layer was confirmed by microscopic examination of the previously catheterized arteries after removal at 44 days.

From the data above, it is particularly preferred that treatment begin with rapamycin or rapamycin plus mycophenolic acid before the procedure is performed, and that treatment should continue after the procedure has been performed. The length of treatment necessary to prevent restenosis will vary from patient to patient. For percutaneous transluminal angioplasty procedures, it is preferred that treatment be administered from 3 or more days before the procedure and continuing for 8 or more days after the procedure. It is more preferred that administration will be for 3 or more days before the angioplasty procedure and continuing for 13 or more days after the procedure. The same administration protocol is applicable when rapamycin, alone or in combination with mycophenolic acid, is used to prevent restenosis following vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures.

When rapamycin is employed alone or in combination with mycophenolic acid in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose

solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin, alone or in combination with mycophenolic acid, may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin, alone or in combination with mycophenolic acid, may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin, alone or in combination with mycophenolic acid can be administered intravascularly or via a vascular stent impregnated with rapamycin, alone or in combination with mycophenolic acid, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin, alone or in combination with mycophenolic acid, may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.001–25 mg/kg, preferably between 0.005–10 mg/kg, and more preferably between 0.01–5 mg/kg. Projected daily oral dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.005–50 mg/kg, preferably between 0.01–25 mg/kg, and more preferably between 0.05–10 mg/kg. Projected daily intravenous dosages of mycophenolic acid, when used in combination with rapamycin, would be 0.5–75 mg/kg and preferably between 5–50 mg/kg. Projected daily oral dosages of mycophenolic acid, when used in combination with rapamycin, would be 1–75 mg/kg and preferably between 10– 50 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, rapamycin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

What is claimed is:

1. A method of treating restenosis in a mammal resulting from said mammal undergoing a percutaneous transluminal coronary angioplasty procedure which comprises administering an antirestenosis effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

2. A method of preventing restenosis in a mammal resulting from said mammal undergoing a percutaneous transluminal coronary angioplasty procedure which comprises administering an antirestenosis effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

3. The method according to claim **2**, wherein the administration of rapamycin is initiated before the mammal undergoes the percutaneous transluminal coronary angioplasty procedure.

4. The method according to claim **3**, wherein the rapamycin is administered for 3 or more days before the mammal undergoes the percutaneous transluminal coronary angioplasty procedure and said administration continues for 8 or more days following the percutaneous transluminal coronary angioplasty procedure.

5. The method according to claim **4**, wherein the rapamycin is administered for 13 or more days following the percutaneous transluminal coronary angioplasty procedure.

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United States Patent [19]

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[54] **METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE**

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[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,288,711.

[21] Appl. No.: **452,051**

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Related U.S. Application Data

[63] Continuation of Ser. No. 238,305, May 12, 1994, Pat. No. 5,516,781, which is a continuation-in-part of Ser. No. 980,000, Nov. 23, 1992, abandoned, which is a continuation of Ser. No. 819,314, Jan. 9, 1992, abandoned.

[51] **Int. Cl.**[6] **A61K 31/71**

[52] **U.S. Cl.** **514/291**; 424/122

[58] **Field of Search** 514/291; 424/122

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[57] **ABSTRACT**

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

4 Claims, No Drawings

METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 08/238,305 filed May 12, 1994, now U.S. Pat. 5,516,781 which is a continuation-in-part of U.S. Ser. No. 07/980,000 filed Nov. 23, 1992, abandoned, which is a continuation of U.S. Ser. No. 07/819,314 filed Jan. 9, 1992, abandoned.

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial lining. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Intimal thickening following arterial injury can be divided into three sequential steps: 1) initiation of smooth muscle cell proliferation following vascular injury, 2) smooth muscle cell migration to the intima, and 3) further proliferation of smooth muscle cells in the intima with deposition of matrix. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A., *Circ. Res.* 56:139 (1985); Clowes, A., J, *Cardiovas. Pharm.* 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9:289 (1989); Forrester, J., *J. Am. Coll. Cardiol.* 17:758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Artherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenisis of artherosclerosis. Artherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P. F.; *Artherosclerosis Lab. Invest.* 55:5 (1986)].

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model

for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others have shown that deendothelilization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S. M., *Human Pathology* 18:240 (1987); Fingerle, J., *Aterioscleroses* 10:1082 (1990)] Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R. G., *JAMA* 265:2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30–40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., *Circulation* 81:1753 (1990); Hardoff, R., *J. Am. Coll. Cardiol.* 15 1486 (1990)].

In an attempt to prevent restenosis or reduce intimal smooth muscle cell proliferation following angioplasty, numerous pharmaceutical agents have been employed clinically, concurrent with or following angioplasty. Most pharmaceutical agents employed in an attempt to prevent or reduce the extent of restenosis have been unsuccessful. The following list identifies several of the agents for which favorable clinical results have been reported: lovastatin [Sahni, R., *Circulation* 80 (Suppl.) 65 (1989); Gellman, J., *J. Am. Coll. Cardiol.* 17:251 (1991)]; thromboxane A_2 synthetase inhibitors such as DP-1904 [Yabe, Y., *Circulation* 80 (Suppl.) 260 (1989)]; eicosapentanoic acid [Nye, E., *Aust. N.Z. J. Med.* 20:549 (1990)]; ciprostene (a prostacyclin analog) [Demke, D., *Brit. J. Haematol* 76 (Suppl.): 20 (1990); Darius, H., *Eur. Heart J.* 12 (Suppl.): 26 (1991)]; trapidil (a platelet derived growth factor) [Okamoto, S., *Circulation* 82 (Suppl.): 428 (1990)]; angiotensin converting enzyme inhibitors [Gottlieb, N., *J. Am. Coll. Cardiol.* 17 (Suppl. A): 181A (1991)]; and low molecular weight heparin [de Vries, C., *Eur. Heart J.* 12 (Suppl.): 386 (1991)].

In an attempt to develop better agents for preventing or reducing smooth muscle proliferation and intimal thickening, the use of balloon catheter induced arterial injury in a variety of mammals has been developed as a standard model of vascular injury that will lead to intimal thickening and eventual vascular narrowing. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. Many compounds have been evaluated in this standard animal model. The immunosuppressive agent cyclosporin A has been evaluated and has produced conflicting results. Jonasson reported that cyclosporin A caused an inhibition of the intimal proliferative lesion following arterial balloon catheterization in vivo, but did not inhibit smooth muscle cell proliferation in vitro. [Jonasson, L., *Proc. Natl. Acad. Sci.* 85:2303 (1988)]. Ferns, however reported that when de-endothelilized rabbits were treated with cyclosporin A, no significant reduction of intimal proliferation was observed in vivo. Additionally, intimal accumulations of foamy macrophages, together with a number of vacuolated smooth muscle cells in the region adjacent to the internal elastic lamina were observed, indicating that cyclosporin A may modify and enhance lesions

that form at the sites of arterial injury. [Ferns, G. A., *Circulation* 80 (Supp): 184 (1989); Ferns, G., *Am. J. Path.* 137:403 (1990)].

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* [U.S. Pat. No. 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., *Can. J. Physiol. Pharm.* 55:48 (1977)], inhibit murine T-cell activation [Staruch, M., *FASEB* 3:3411 (1989)], prolong survival time of organ grafts in histoincompatible rodents [Morris, R., *Med. Sci. Res.* 17:877 (1989)], and inhibit transplantation rejection in mammals [Calne, R., European Patent Application 401,747]. Rapamycin blocks calcium-dependent, calcium-independent, cytokine-independent and constitutive T and B cell division at the G1-S interface. Rapamycin inhibits gamma-interferon production induced by Il-1 and also inhibits the gamma-interferon induced expression of membrane antigen. [Morris, R. E., *Transplantation Rev.* 6:39 (1992)]. The use of rapamycin in preventing coronary graft atherosclerosis (CGA) in rats has been disclosed by Meiser [*J. Heart Lung Transplant* 9:55 (1990)]. Arterial thickening following transplantation, known as CGA, is a limiting factor in graft survival that is caused by a chronic immunological response to the transplanted blood vessels by the transplant recipient's immune system. [Dec. G, *Transplantation Proc.* 23:2095 (1991) and Dunn, M. *Lancet* 339:1566 (1992)]. The disclosed invention is distinct from the use of rapamycin for preventing CGA, in that CGA does not involve injury to the recipients own blood vessels; it is a rejection type response. The disclosed invention is related to vascular injury to native blood vessels. The resulting intimal smooth muscle cell proliferation dose not involve the immune system, but is growth factor mediated. For example, arterial intimal thickening after balloon catheter injury is believed to be caused by growth factor (PGDF, bFGF, TGFb, IL-1 and others)-induced smooth muscle cell proliferation and migration. [Ip, J. H., *J. Am. Coll. Cardiol* 15:1667 (1990)]. Ferns has also shown that the immune response is not involved in arterial intimal thickening following balloon catheterization, as he found that there was no difference in intimal thickening between arteries from athymic nude rats (rats lacking T-cells) and normal rats after balloon catheterization [*Am. J. Pathol.* 138:1045 (1991)].

DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium. Rapamycin is also useful in preventing intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion resulting from mechanically mediated injury. In particular, for the prevention of restenosis following a percutaneous transluminal coronary angioplasty procedure.

Treating includes retarding the progression, arresting the development, as well as palliation. Preventing includes inhibiting the development of and prophylacticly preventing of hyperproliferative vascular disease in a susceptible mammal.

This invention also provides a method of using a combination of rapamycin and mycophenolic acid for the same utilities described above. Mycophenolic acid, an antiproliferative antimetabolite, inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, enzymes in the de novo purine biosynthetic pathway. This results in an inhibition of DNA synthesis which causes an accumulation of cells at the G1-S interface. Other combinations containing rapamycin that are useful for preventing or treating hyperproliferative vascular disease will be apparent to one skilled in the art. These include, but are not limited to, using rapamycin in combination with other antiproliferative antimetabolites.

The effect of rapamycin on hyperproliferative vascular disease was established in an in vitro and an in vivo standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. Cycloporin A was also evaluated in these test procedures for the purpose of comparison. The combination of rapamycin and mycophenolic acid was evaluated in the in vivo test procedure. The procedures and the results obtained are described below.

Rapamycin and cyclosporin A were evaluated in an in vitro standard pharmacological test procedure which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Results were obtained by measuring DNA and protein synthesis in rat smooth muscle cells that have been stimulated with a growth factor such as fetal calf serum or a hypertrophic mitogen, such as angiotensin II. The following briefly describes the procedure that was used. Rat smooth muscle cells were maintained in a 1:1 mixture of defined Eagle's medium (DEM) and Ham's F12 medium with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL) and 25 mL Hepes at pH 7.4. Cells were incubated at 37° C. in a humidified atmosphere of 5% CO_2 with media changes every 2–3 days. Each compound tested was diluted with an appropriate vehicle to obtain a 1 mM stock solution. Ethanol was used as the vehicle for rapamycin and 20% tween 80 in ethanol was the vehicle for cyclosporin A. Test concentrations of drug were obtained by diluting appropriate concentrations of stock solution with serum free media. The smooth muscle cell culture was maintained in a defined serum free media containing 1:1 DEM and Ham's F12 medium, insulin (5×10^{-7}M), transferrin (5 μg/mL), and ascorbate (0.2 mM) for 72 hours before testing in a multi-well plate. After the 72 hour period, an appropriate quantity of stock solution containing either rapamycin or cyclosporin A was added to the smooth muscle cell culture and media mixture. After a 24 hours the appropriate growth factor was added. For the measurement of DNA synthesis, ^{3}H-thymidine was added at 12 hours after the growth factor was added, and the cells were harvested at 36 hours. For the measurement of protein synthesis, ^{3}H-leucine was added at 14 hours after the growth factor was added, and the cells were harvested at 18 hours. The amount

of incorporated radioactive label was measured on a scintillation counter.

The following table shows the results obtained for rapamycin on DNA and protein synthesis in smooth muscle cells that were stimulated with 10% fetal calf serum, as measured by incorporation of tritiated thymidine or leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only was normalized to 100%, and the results for cells treated with fetal calf serum or fetal calf serum plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON DNA AND PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH FETAL CALF SERUM*

	^{3}H-Thymidine Incorporation (% of Media)	^{3}H-Leucine Incorporation (% of Media)
Media	100%	100%
FCS	495%	174%
1000 nM RAP + FCS	136%	95%
100 nM RAP + FCS	172%	91%
10 nM RAP + FCS	204%	74%
1 nM RAP + FCS	403%	106%

*Abbreviations: RAP = rapamycin; Media = defined serum free media; and FCS = 10% fetal calf serum.

The following table shows the results obtained for rapamycin on protein synthesis in smooth muscle cells that were stimulated with 10^{-6} nM angiotensin II, as measured by incorporation of tritiated leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only were normalized to 100%, and the results for cells treated with angiotensin or angiotensin plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH ANGIOTENSIN II*

	^{3}H-Leucine Incorporation (% of Media)
Media	100%
ANG	159%
1000 nM RAP + ANG	53%
100 nM RAP + ANG	57%
10 nM RAP + ANG	61%
1 nM RAP + ANG	60%

*Abbreviations: RAP = rapamycin; Media = defined serum free media; and ANG = 10^{-6} nM angiotensin II.

The results of the standard in vitro test procedure showed that rapamycin had a pronounced antiproliferative effect in the presence of FCS and an anti-hypertrophic effect in the presence of angiotensin II. Following vascular injury, DNA and protein synthesis of smooth muscle cells are necessary for the development of restenosis to occur. These results showed that rapamycin inhibited both DNA and protein synthesis in stimulated smooth muscle cells. An antiproliferative effect was also observed with cyclosporin A; however, at 1000 nM, cyclosporin A was cytotoxic and not merely cytostatic. At 1000 nM, cyclosporin A caused lysis of the smooth muscle cells as evidenced by the presence of lactic acid dehydrogenase in the supernatant of the cell culture. Similar toxicity to smooth muscle cells was not observed for rapamycin.

Rapamycin, rapamycin plus mycophenolic acid, and cyclosporin A were evaluated in an in vivo standard pharmacological test procedure that emulates the vascular injury suffered and restenosis that develops following percutaneous transluminal coronary angioplasty in humans. The ability of a test compound to inhibit restenosis was determined by comparing intimal thickening in mammals treated with test compound following balloon catheterization versus intimal thickening in untreated control mammals after the same test procedure. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. The following briefly describes the procedure that was used. The left carotid arteries of male Sprague-Dawley rats were injured with an inflated 2 Fr balloon catheter. During a 14 day postoperative period, these rats were divided into groups and treated daily with rapamycin (1.5 mg/kg; i.p.), rapamycin plus mycophenolic acid (1.5 mg/kg; i.p.+40 mg/kg; p.o.), or cyclosporin A (3 mg/kg; i.p.). Treatment was administered on days 0 to 13 postoperatively. Additionally, one group each also received rapamycin (6 mg/kg/day; i.p.) or cyclosporin A (40 mg/kg/day; i.p.) for two days postoperatively, and then received no treatment for the next 12 days. An untreated group was used an injured control to establish the amount of intimal growth in the absence of treatment. The fight carotid was used as an uninjured control in all groups. After the 14-day period, the rats were sacrificed, the carotids removed. The mean areas of the intima and blood vessel wall were measured by morphometry. Results are expressed as an intima percent which can be expressed according to the following formula:

$$\frac{\text{area of intima}}{\text{area of vessel}} \times 100$$

The following table shows the results that were obtained.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)*

Test Group	Intima Percent ± S.E.
Uninjured Control	0.00 ± 0.00
Untreated Injured Control	33.3 ± 19.66
RAP (1.5 mg/kg - 14 days)	6.78 ± 4.69
RAP (6 mg/kg - 2 days)	16.56 ± 6.22
RAP + MPA (14 days)	1.6 ± 3.5
CsA (3 mg/kg - 14 days)	26.46 ± 27.42
CsA (40 mg/kg - 2 days)	31.14 ± 20.66

*Abbreviations RAP = rapamycin; MPA = mycophenolic acid; and CsA = cyclosporin A.

These results show that treatment with rapamycin (1.5 mg/kg for 14 days) resulted in an 80% decrease in the mean percentage intimal thickening compared with the untreated injured control group. Similarly, treatment with the combination of rapamycin and mycophenolic acid produced almost a complete inhibition of intimal thickening (95% reduction in intimal thickening compared with untreated injured control). Cyclosporin A failed to produce any meaningful reduction in intimal thickening.

Similar results were obtained when rapamycin was evaluated at different doses in the above in vivo standard pharmacological test procedure that emulates the vascular injury that occurs following a percutaneous transluminal coronary angioplasty procedure in humans. Rapamycin was administered on postoperative days 0–13, and examination by

morphometry was performed on day 14. Rapamycin, at a dose of 1.5 and 3 mg/kg significantly arrested the development of restenosis as measured by the intima percent 14 days after balloon catheterization, whereas restenosis was clearly observed in the untreated injured control group. These results are summarized in the table below.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	6 mg/kg	0–13	30.92 ± 4.06
Rapamycin	3 mg/kg	0–13	22.68 ± 6.28
Rapamycin	1.5 mg/kg	0–13	21.89 ± 4.2

The results of the in vitro and in vivo standard test procedures demonstrate that rapamycin and rapamycin in combination with mycophenolic acid are useful in treating hyperproliferative vascular disease.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Rapamycin and rapamycin plus mycophenolic acid were also evaluated in a modification of the in vivo test procedure described above. In the modified test procedure, treatment with rapamycin or rapamycin plus mycophenolic acid were stopped on day 14, as above, but the animals were not sacrificed immediately. Intimal thickening was observed when the animals were sacrificed 1, 2, 4 weeks, and 44 days after treatment had been stopped. Microscopic analysis showed that endothelium regeneration had not occurred during the two week treatment period. For example, 44 days after undergoing balloon catheterization procedure of the carotid artery, untreated injured control rats had an intima percent (±S.E.) of 62.85±3.63, and rats treated with rapamycin+mycophenolic acid (1.5/40 mg/kg) on postoperative days 0–13 had an intima percent (±S.E.) of 50.39±2.58. Better results were not obtained when the same regimen was administered on days 0–30 (intima percent (±S.E.) of 53.55±2.85). Following cessation of treatment with rapamycin or rapamycin plus mycophenolic acid intimal proliferation, that was previously suppressed, was able to occur. These results are consistent with the results shown in the table above, in which treatment for 2 days with rapamycin followed by 12 days of no treatment inhibited intimal thickening to a lesser degree than treatment with rapamycin for 14 days. These results are expected, as in the absence on an integral endothelial layer, the intimal smooth muscle cells will proliferate. It has been shown that intimal smooth muscle cell growth does not have an inhibitory effect on normal endothelial regeneration, and that intimal smooth muscle cell proliferation ceases when the endothelial layer is established. [Reidy, M., *Lab. Invest.* 59:36 (1988); Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979)]. As such, treatment with rapamycin or rapamycin in combination with mycophenolic acid should be employed so long as the beneficial effect is seen. As the degree of restenosis can be monitored by angiographic and sonographic techniques, the dosage necessary to sustain the opened vessels can be adjusted.

To evaluate the ability of rapamycin and rapamycin plus mycophenolic acid to prevent restenosis following an angioplasty procedure, rapamycin was evaluated in the same in vivo standard pharmacological test procedure for restenosis that was described above, except that treatment with rapamycin began three days before (day −3) the angioplasty procedure was performed. The following table shows the results obtained on day 14 following balloon catheterization of the carotid artery on day 0. Results for treatment from day 3 to 13 are also provided.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	1.5 mg/kg	−3–13*	9.85 ± 1.15
Rapamycin	1.5 mg/kg	−3–3	30.7 ± 6.67
Rapamycin	1.5 mg/kg	−3–0	37.31 ± 4.33
Rapamycin	1.5 mg/kg	3–13	44.38 ± 5.49

*Treatment from three days pre-balloon catheterization to day 13 days post-catheterization.

The results in the table above show that rapamycin prevented the development of restenosis following a balloon angioplasty procedure of the carotid artery, when rapamycin was administered from three days pre-angioplasty until day 13. Treatment from day minus 3 until day 3 or day 0 afforded a lesser degree of prevention, and treatment from day 3 to day 13 did not prevent restenosis.

The effect of rapamycin plus mycophenolic acid (MPA) was also evaluated in the angioplasty standard pharmacological test procedure. The table below shows the results obtained where rats underwent a balloon catheterization procedure of the carotid artery on day 0, and were sacrificed and examined morphometrically on day 44. The treatment regimen is described in the table.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			62.85 ± 3.63
Rapamycin + MPA	40/1.5 mg/kg	0–13	50.39 ± 2.58
Rapamycin + MPA	40/1.5 mg/kg	0–30	53.55 ± 2.85
Rapamycin + MPA	40/1.5 mg/kg	−3–13	18.76 ± 10.6

These results show that treatment with rapamycin and mycophenolic acid from day minus 3 to day 13 did effec-

tively prevent restenosis at day 44, whereas the regimens which did not include drug administration before the angioplasty procedure did not effectively prevent restenosis at day 44.

Similar results were obtained when rat thoracic aortas were subjected to a balloon catheterization procedure, as described above, on day 0. The rats were either sacrificed and examined on day 14 or on day 44. The results obtained with rapamycin and rapamycin plus mycophenolic acid (MPA) are shown in the table below.

EFFECT OF RAPAMYCIN AND RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED THORACIC AORTAS

Group	Dose	Treatment Days	Intima Percent ± S.E.
	Day 14 results		
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			15.52 ± 2.99
Rapamycin + MPA	40/1.5 mg/kg	−3–13	0.00 ± 0.00
	Day 44 Results		
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			28.76 ± 6.52
Rapamycin	1.5 mg/kg	−3–13	0.00 ± 0.00
Rapamycin + MPA	40/1.5 mg/kg	−3–13	8.76 ± 3.34

The results in the table above show that treatment with rapamycin from 3 days preoperatively until 13 days postoperatively completely prevented the development of restenosis 44 days after a balloon catheterization of the thoracic aorta. Using the same treatment regimen, rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterization and significantly prevented restenosis 44 days following balloon catheterization.

Similarly, day minus 3 to day 13 treatment with rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterizaton of the abdominal aortas in rats. These results are shown in the table below.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED ABDOMINAL AORTAS (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			10.17 ± 2.42
Rapamycin + MPA	40/1.5 mg/kg	−3–13	0.00 ± 0.00

The results in the tables above show that rapamycin, alone or in combination with mycophenolic acid, is useful in preventing restenosis following invasive procedures that disrupt the vascular endothelial lining, such as percutaneous transluminal coronary angioplasty, vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures. These data also show that the administration of rapamycin, alone or in combination with mycophenolic acid, from 3 days pre-catheterization to 13 days post-catheterization, allowed the endothelium to heal, while preventing intimal smooth muscle cell proliferation. That intimal proliferation did not occur 31 days after administration with rapamycin, alone or in combination with mycophenolic acid, had been stopped, demonstrates that the endothelial layer had regenerated, as intimal proliferation stops after the reestablishment of the endothelial layer. The reestablishment of an intact endothelial layer was confirmed by microscopic examination of the previously catheterized arteries after removal at 44 days.

From the data above, it is particularly preferred that treatment begin with rapamycin or rapamycin plus mycophenolic acid before the procedure is performed, and that treatment should continue after the procedure has been performed. The length of treatment necessary to prevent restenosis will vary from patient to patient. For percutaneous transluminal angioplasty procedures, it is preferred that treatment be administered from 3 or more days before the procedure and continuing for 8 or more days after the procedure. It is more preferred that administration will be for 3 or more days before the angioplasty procedure and continuing for 13 or more days after the procedure. The same administration protocol is applicable when rapamycin, alone or in combination with mycophenolic acid, is used to prevent restenosis following vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures.

When rapamycin is employed alone or in combination with mycophenolic acid in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example,

intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin, alone or in combination with mycophenolic acid, may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin, alone or in combination with mycophenolic acid, may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin, alone or in combination with mycophenolic acid can be administered intravascularly or via a vascular stent impregnated with rapamycin, alone or in combination with mycophenolic acid, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin, alone or in combination with mycophenolic acid, may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.001–25 mg/kg, preferably between 0.005–10 mg/kg, and more preferably between 0.01–5 mg/kg. Projected daily oral dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.005–50 mg/kg, preferably between 0.01–25 mg/kg, and more preferably between 0.05–10 mg/kg. Projected daily intravenous dosages of mycophenolic acid, when used in combination with rapamycin, would be 0.5–75 mg/kg and preferably between 5–50 mg/kg. Projected daily oral dosages of mycophenolic acid, when used in combination with rapamycin, would be 1–75 mg/kg and preferably between 10–50 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, rapamycin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

What is claimed is:

1. A method of preventing restenosis in a mammal resulting from said mammal undergoing a vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedure which comprises administering an antirestenosis effective amount of rapamycin to said mammal orally, parenterally intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

2. The method according to claim **1**, wherein the administration of the rapamycin is initiated before the mammal undergoes the procedure.

3. The method according to claim **2**, wherein the rapamycin is administered for 3 or more days before the mammal undergoes the procedure and said administration continues for 8 or more days following the procedure.

4. The method according to claim **3**, wherein the rapamycin is administered for 13 or more days following the procedure.

* * * * *

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United States Patent [19]

Morris et al.

[11] Patent Number: 5,665,728

[45] Date of Patent: *Sep. 9, 1997

[54] **METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE**

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[73] Assignee: **American Home Products Corporation**, Madison, N.J.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,288,711.

[21] Appl. No.: **451,627**

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Related U.S. Application Data

[63] Continuation of Ser. No. 238,305, May 12, 1994, Pat. No. 5,516,781, which is a continuation-in-part of Ser. No. 980,000, Nov. 23, 1992, abandoned, which is a continuation of Ser. No. 819,314, Jan. 9, 1992, abandoned.

[51] **Int. Cl.**[6] **A61K 31/345**

[52] **U.S. Cl.** **514/291**; 424/122

[58] **Field of Search** 514/291; 424/122

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[57] **ABSTRACT**

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

2 Claims, No Drawings

METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 08/238,305 filed May 12, 1994, now U.S. Pat. No. 5,561,781 which is a continuation-in-part of U.S. Ser. No. 07/980,000 filed Nov. 23, 1992, now abandoned which is a continuation of U.S. Ser. No. 07/819,314 filed Jan. 9, 1992 now abandoned.

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial lining. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Intimal thickening following arterial injury can be divided into three sequential steps: 1) initiation of smooth muscle cell proliferation following vascular injury, 2) smooth muscle cell migration to the intima, and 3) further proliferation of smooth muscle cells in the intima with deposition of matrix. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A., *Circ. Res.* 56:139 (1985); Clowes, A., J, *Cardiovas. Pharm.* 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9:289 (1989); Forrester, J., *J. Am. Coll. Cardiol.* 17:758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Artherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenisis of artherosclerosis. Artherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P. F., *Artherosclerosis Lab. Invest.* 55:5 (1986)].

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others have shown that deendothelilization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S. M., *Human Pathology* 18:240 (1987); Fingerle, J., *Aterioscleroses* 10:1082 (1990)] Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R. G., *JAMA* 265:2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30–40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., *Circulation* 81:1753 (1990); Hardoff, R., *J. Am. Coll. Cardiol.* 15 1486 (1990)].

In an attempt to prevent restenosis or reduce intimal smooth muscle cell proliferation following angioplasty, numerous pharmaceutical agents have been employed clinically, concurrent with or following angioplasty. Most pharmaceutical agents employed in an attempt to prevent or reduce the extent of restenosis have been unsuccessful. The following list identifies several of the agents for which favorable clinical results have been reported: lovastatin [Sahni, R., *Circulation* 80 (Suppl.) 65 (1989); Gellman, J., *J. Am. Coll. Cardiol.* 17:251 (1991)]; thromboxane A_2 synthetase inhibitors such as DP-1904 [Yabe, Y., *Circulation* 80 (Suppl.) 260 (1989)]; eicosapentanoic acid [Nye, E., *Aust. N. Z. J. Med.* 20:549 (1990)]; ciprostene (a prostacyclin analog) [Demke, D., *Brit. J. Haematol* 76 (Suppl.): 20 (1990); Darius, H., *Eur. Heart J.* 12 (Suppl.): 26 (1991)]; trapidil (a platelet derived growth factor) [Okamoto, S., *Circulation* 82 (Suppl.): 428 (1990)]; angiotensin converting enzyme inhibitors [Gottlieb, N., *J. Am. Coll. Cardiol.* 17 (Suppl. A): 181A (1991)]; and low molecular weight heparin [de Vries, C., *Eur. Heart J.* 12 (Suppl.): 386 (1991)].

In an attempt to develop better agents for preventing or reducing smooth muscle proliferation and intimal thickening, the use of balloon catheter induced arterial injury in a variety of mammals has been developed as a standard model of vascular injury that will lead to intimal thickening and eventual vascular narrowing. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. Many compounds have been evaluated in this standard animal model. The immunosuppressive agent cyclosporin A has been evaluated and has produced conflicting results. Jonasson reported that cyclosporin A caused an inhibition of the intimal proliferative lesion following arterial balloon catheterization in vivo, but did not inhibit smooth muscle cell proliferation in vitro. [Jonasson, L., *Proc. Natl. Acad. Sci.* 85:2303 (1988)]. Ferns, however reported that when de-endothelilized rabbits were treated with cyclosporin A, no significant reduction of intimal proliferation was observed in vivo. Additionally, intimal accumulations of foamy macrophages, together with a number of vacuolated smooth muscle cells in the region adjacent to the internal elastic lamina were observed, indicating that cyclosporin A may modify and enhance lesions that form at the sites of arterial injury. [Ferns, G. A.,

Circulation 80 (Supp): 184 (1989); Ferns, G., *Am. J. Path.* 137:403 (1990)].

Rapamycin, a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus* [U.S. Pat. No. 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., *Can. J. Physiol. Pharm.* 55:48 (1977)], inhibit murine T-cell activation [Staruch, M., *FASEB* 3:3411 (1989)], prolong survival time of organ grafts in histoincompatible rodents [Morris, R., *Med. Sci. Res.* 17:877 (1989)], and inhibit transplantation rejection in mammals [Calne, R., European Patent Application 401,747]. Rapamycin blocks calcium-dependent, calcium-independent, cytokine-independent and constitutive T and B cell division at the G1-S interface. Rapamycin inhibits gamma-interferon production induced by I1-1 and also inhibits the gamma-interferon induced expression of membrane antigen. [Morris, R. E., *Transplantation Rev.* 6:39 (1992)]. The use of rapamycin in preventing coronary graft atherosclerosis (CGA) in rats has been disclosed by Meiser [*J. Heart Lung Transplant* 9:55 (1990)]. Arterial thickening following transplantation, known as CGA, is a limiting factor in graft survival that is caused by a chronic immunological response to the transplanted blood vessels by the transplant recipient's immune system. [Dec. G, *Transplantation Proc.* 23:2095 (1991) and Dunn, M. *Lancet* 339:1566 (1992)]. The disclosed invention is distinct from the use of rapamycin for preventing CGA, in that CGA does not involve injury to the recipients own blood vessels; it is a rejection type response. The disclosed invention is related to vascular injury to native blood vessels. The resulting intimal smooth muscle cell proliferation dose not involve the immune system, but is growth factor mediated. For example, arterial intimal thickening after balloon catheter injury is believed to be caused by growth factor (PGDF, bFGF, TGFb, IL-1 and others)-induced smooth muscle cell proliferation and migration. [Ip, J. H., *J. Am. Coll. Cardiol* 15:1667 (1990)]. Ferns has also shown that the immune response is not involved in arterial intimal thickening following balloon catheterization, as he found that there was no difference in intimal thickening between arteries from athymic nude rats (rats lacking T-cells) and normal rats after balloon catheterization [*Am. J. Pathol.* 138:1045 (1991)].

DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium. Rapamycin is also useful in preventing intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion resulting from mechanically mediated injury. In particular, for the prevention of restenosis following a percutaneous transluminal coronary angioplasty procedure.

Treating includes retarding the progression, arresting the development, as well as palliation. Preventing includes inhibiting the development of and prophylacticly preventing of hyperproliferative vascular disease in a susceptible mammal.

This invention also provides a method of using a combination of rapamycin and mycophenolic acid for the same utilities described above. Mycophenolic acid, an antiproliferative antimetabolite, inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, enzymes in the de novo purine biosynthetic pathway. This results in an inhibition of DNA synthesis which causes an accumulation of cells at the G1-S interface. Other combinations containing rapamycin that are useful for preventing or treating hyperproliferative vascular disease will be apparent to one skilled in the art. These include, but are not limited to, using rapamycin in combination with other antiproliferative antimetabolites.

The effect of rapamycin on hyperproliferative vascular disease was established in an in vitro and an in vivo standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. Cycloporin A was also evaluated in these test procedures for the purpose of comparison. The combination of rapamycin and mycophenolic acid was evaluated in the in vivo test procedure. The procedures and the results obtained are described below.

Rapamycin and cyclosporin A were evaluated in an in vitro standard pharmacological test procedure which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Results were obtained by measuring DNA and protein synthesis in rat smooth muscle cells that have been stimulated with a growth factor such as fetal calf serum or a hypertrophic mitogen, such as angiotensin II. The following briefly describes the procedure that was used. Rat smooth muscle cells were maintained in a 1:1 mixture of defined Eagle's medium (DEM) and Ham's F12 medium with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL) and 25 mL Hepes at pH 7.4. Cells were incubated at 37° C. in a humidified atmosphere of 5% CO_2 with media changes every 2–3 days. Each compound tested was diluted with an appropriate vehicle to obtain a 1 mM stock solution. Ethanol was used as the vehicle for rapamycin and 20% tween 80 in ethanol was the vehicle for cyclosporin A. Test concentrations of drug were obtained by diluting appropriate concentrations of stock solution with serum free media. The smooth muscle cell culture was maintained in a defined serum free media containing 1:1 DEM and Ham's F12 medium, insulin (5×10^{-7}M), transferrin (5 μg/mL), and ascorbate (0.2 mM) for 72 hours before testing in a multi-well plate. After the 72 hour period, an appropriate quantity of stock solution containing either rapamycin or cyclosporin A was added to the smooth muscle cell culture and media mixture. After a 24 hours the appropriate growth factor was added. For the measurement of DNA synthesis, ^{3}H-thymidine was added at 12 hours after the growth factor was added, and the cells were harvested at 36 hours. For the measurement of protein synthesis, ^{3}H-leucine was added at 14 hours after the growth factor was added, and the cells were harvested at 18 hours. The amount of incorporated radioactive label was measured on a scintillation counter.

The following table shows the results obtained for rapamycin on DNA and protein synthesis in smooth muscle cells that were stimulated with 10% fetal calf serum, as measured by incorporation of tritiated thymidine or leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only was normalized to 100%, and the results for cells treated with fetal calf serum or fetal calf serum plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON DNA AND PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH FETAL CALF SERUM*

	^{3}H-Thymidine Incorporation (% of Media)	^{3}H-Leucine Incorporation (% of Media)
Media	100%	100%
FCS	495%	174%
1000 nM RAP + FCS	136%	95%
100 nM RAP + FCS	172%	91%
10 nM RAP + FCS	204%	74%
1 nM RAP + FCS	403%	106%

*Abbreviations: RAP = rapamycin; Media = defined serum free media; and FCS = 10% fetal calf serum.

The following table shows the results obtained for rapamycin on protein synthesis in smooth muscle cells that were stimulated with 10^{-6} nM angiotensin II, as measured by incorporation of tritiated leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only were normalized to 100%, and the results for cells treated with angiotensin or angiotensin plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH ANGIOTENSIN II*

	^{3}H-Leucine Incorporation (% of Media)
Media	100%
ANG	159%
1000 nM RAP + ANG	53%
100 nM RAP + ANG	57%
10 nM RAP + ANG	61%
1 nM RAP + ANG	60%

*Abbreviations: RAP = rapamycin; Media = defined serum free media; and ANG = 10^{-6} nM angiotensin II.

The results of the standard in vitro test procedure showed that rapamycin had a pronounced antiproliferative effect in the presence of FCS and an anti-hypertrophic effect in the presence of angiotensin II. Following vascular injury, DNA and protein synthesis of smooth muscle cells are necessary for the development of restenosis to occur. These results showed that rapamycin inhibited both DNA and protein synthesis in stimulated smooth muscle cells. An antiproliferative effect was also observed with cyclosporin A; however, at 1000 nM, cyclosporin A was cytotoxic and not merely cytostatic. At 1000 nM, cyclosporin A caused lysis of the smooth muscle cells as evidenced by the presence of lactic acid dehydrogenase in the supernatant of the cell culture. Similar toxicity to smooth muscle cells was not observed for rapamycin.

Rapamycin, rapamycin plus mycophenolic acid, and cyclosporin A were evaluated in an in vivo standard pharmacological test procedure that emulates the vascular injury suffered and restenosis (hat develops following percutaneous transluminal coronary angioplasty in humans. The ability of a test compound to inhibit restenosis was determined by comparing intimal thickening in mammals treated with test compound following balloon catheterization versus intimal thickening in untreated control mammals after the same test procedure. [Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979); Clowes, A. W., *Lab. Invest.* 49:208 (1983); Clowes, A. W., *J. Cardiovas. Pharm.* 14:S12 (1989); and Ferns, G. A., *Science* 253:1129 (1991)]. The following briefly describes the procedure that was used. The left carotid arteries of male Sprague-Dawley rats were injured with an inflated 2 Fr balloon catheter. During a 14 day postoperative period, these rats were divided into groups and treated daily with rapamycin (1.5 mg/kg; i.p.), rapamycin plus mycophenolic acid (1.5 mg/kg; i.p. +40 mg/kg; p.o.), or cyclosporin A (3 mg/kg; i.p.). Treatment was administered on days 0 to 13 postoperatively. Additionally, one group each also received rapamycin (6 mg/kg/day; i.p.) or cyclosporin A (40 mg/kg/day; i.p.) for two days postoperatively, and then received no treatment for the next 12 days. An untreated group was used an injured control to establish the amount of intimal growth in the absence of treatment. The right carotid was used as an uninjured control in all groups. After the 14-day period, the rats were sacrificed, the carotids removed. The mean areas of the intima and blood vessel wall were measured by morphometry. Results are expressed as an intima percent which can be expressed according to the following formula:

$$\frac{\text{area of intima}}{\text{area of vessel}} * 100$$

The following table shows the results that were obtained.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTIID ARTERIES (DAY 14)*

Test Group	Intima Percent ± S.E.
Uninjured Control	0.00 ± 0.00
Untreated Injured Control	33.3 ± 19.66
RAP (1.5 mg/kg - 14 days)	6.78 ± 4.69
RAP (6 mg/kg - 2 days)	16.56 ± 6.22
RAP + MPA (14 days)	1.6 ± 3.5
CsA (3 mg/kg - 14 days)	26.46 ± 27.42
CsA (40 mg/kg - 2 days)	31.14 ± 20.66

*Abbreviations RAP = rapamycin; MPA = mycophenolic acid; and CsA = cyclosporin A.

These results show that treatment with rapamycin (1.5 mg/kg for 14 days) resulted in an 80% decrease in the mean percentage intimal thickening compared with the untreated injured control group. Similarly, treatment with the combination of rapamycin and mycophenolic acid produced almost a complete inhibition of intimal thickening (95% reduction in intimal thickening compared with untreated injured control). Cyclosporin A failed to produce any meaningful reduction in intimal thickening.

Similar results were obtained when rapamycin was evaluated at different doses in the above in vivo standard pharmacological test procedure that emulates the vascular injury that occurs following a percutaneous transluminal coronary angioplasty procedure in humans. Rapamycin was administered on postoperative days 0–13, and examination by morphometry was performed on day 14. Rapamycin, at a dose of 1.5 and 3 mg/kg significantly arrested the develop-

ment of restenosis as measured by the intima percent 14 days after balloon catheterization, whereas restenosis was clearly observed in the untreated injured control group. These results are summarized in the table below.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	6 mg/kg	0–13	30.92 ± 4.06
Rapamycin	3 mg/kg	0–13	22.68 ± 6.28
Rapamycin	1.5 mg/kg	0–13	21.89 ± 4.2

The results of the in vitro and in vivo standard test procedures demonstrate that rapamycin and rapamycin in combination with mycophenolic acid are useful in treating hyperproliferative vascular disease.

As such, rapamycin is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Rapamycin and rapamycin plus mycophenolic acid were also evaluated in a modification of the in vivo test procedure described above. In the modified test procedure, treatment with rapamycin or rapamycin plus mycophenolic acid were stopped on day 14, as above, but the animals were not sacrificed immediately. Intimal thickening was observed when the animals were sacrificed 1, 2, 4 weeks, and 44 days after treatment had been stopped. Microscopic analysis showed that endothelium regeneration had not occurred during the two week treatment period. For example, 44 days after undergoing balloon catheterization procedure of the carotid artery, untreated injured control rats had an intima percent (±S.E.) of 62.85±3.63, and rats treated with rapamycin+mycophenolic acid (1.5/40 mg/kg) on postoperative days 0–13 had an intima percent (±S.E.) of 50.39±2.58. Better results were not obtained when the same regimen was administered on days 0–30 (intima percent (±S.E.) of 53.55±2.85). Following cessation of treatment with rapamycin or rapamycin plus mycophenolic acid intimal proliferation, that was previously suppressed, was able to occur. These results are consistent with the results shown in the table above, in which treatment for 2 days with rapamycin followed by 12 days of no treatment inhibited intimal thickening to a lesser degree than treatment with rapamycin for 14 days. These results are expected, as in the absence on an integral endothelial layer, the intimal smooth muscle cells will proliferate. It has been shown that intimal smooth muscle cell growth does not have an inhibitory effect on normal endothelial regeneration, and that intimal smooth muscle cell proliferation ceases when the endothelial layer is established. [Reidy, M., *Lab. Invest.* 59:36 (1988); Chevru, A., *Surg. Gynecol. Obstet.* 171:443 (1990); Fishman, J., *Lab. Invest.* 32:339 (1975); Haudenschild, C., *Lab. Invest.* 41:407 (1979)]. As such, treatment with rapamycin or rapamycin in combination with mycophenolic acid should be employed so long as the beneficial effect is seen. As the degree of restenosis can be monitored by angiographic and sonographic techniques, the dosage necessary to sustain the opened vessels can be adjusted.

To evaluate the ability of rapamycin and rapamycin plus mycophenolic acid to prevent restenosis following an angioplasty procedure, rapamycin was evaluated in the same in vivo standard pharmacological test procedure for restenosis that was described above, except that treatment with rapamycin began three days before (day –3) the angioplasty procedure was performed. The following table shows the results obtained on day 14 following balloon catheterization of the carotid artery on day 0. Results for treatment from day 3 to 13 are also provided.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			44.51 ± 5.03
Rapamycin	1.5 mg/kg	–3–13*	9.85 ± 1.15
Rapamycin	1.5 mg/kg	–3–3	30.7 ± 6.67
Rapamycin	1.5 mg/kg	–3–0	37.31 ± 4.33
Rapamycin	1.5 mg/kg	3–13	44.38 ± 5.49

*Treatment from three days pre-balloon catheterization to day 13 days post-catheterization.

The results in the table above show that rapamycin prevented the development of restenosis following a balloon angioplasty procedure of the carotid artery, when rapamycin was administered from three days pre-angioplasty until day 13. Treatment from day minus 3 until day 3 or day 0 afforded a lesser degree of prevention, and treatment from day 3 to day 13 did not prevent restenosis.

The effect of rapamycin plus mycophenolic acid (MPA) was also evaluated in the angioplasty standard pharmacological test procedure. The table below shows the results obtained where rats underwent a balloon catheterization procedure of the carotid artery on day 0, and were sacrificed and examined morphometrically on day 44. The treatment regimen is described in the table.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES (DAY 44)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			62.85 ± 3.63
Rapamycin + MPA	40/1.5 mg/kg	0–13	50.39 ± 2.58
Rapamycin + MPA	40/1.5 mg/kg	0–30	53.55 ± 2.85
Rapamycin + MPA	40/1.5 mg/kg	–3–13	18.76 ± 10.6

These results show that treatment with rapamycin and mycophenolic acid from day minus 3 to day 13 did effectively prevent restenosis at day 44, whereas the regimens which did not include drug administration before the angioplasty procedure did not effectively prevent restenosis at day 44.

Similar results were obtained when rat thoracic aortas were subjected to a balloon catheterization procedure, as described above, on day 0. The rats were either sacrificed and examined on day 14 or on day 44. The results obtained with rapamycin and rapamycin plus mycophenolic acid (MPA) are shown in the table below.

EFFECT OF RAPAMYCIN AND RAPAMYCIN + MPA ON THICKENING IN INJURED THORACIC AORTAS

Group	Dose	Treatment Days	Intima Percent ± S.E.
Day 14 results			
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			15.52 ± 2.99
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00
Day 44 Results			
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			28.76 ± 6.52
Rapamycin	1.5 mg/kg	–3–13	0.00 ± 0.00
Rapamycin + MPA	40/1.5 mg/kg	–3–13	8.76 ± 3.34

The results in the table above show that treatment with rapamycin from 3 days preoperatively until 13 days postoperatively completely prevented the development of restenosis 44 days after a balloon catheterization of the thoracic aorta. Using the same treatment regimen, rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterization and significantly prevented restenosis 44 days following balloon catheterization.

Similarly, day minus 3 to day 13 treatment with rapamycin plus mycophenolic acid completely prevented restenosis 14 days after balloon catheterizaton of the abdominal aortas in rats. These results are shown in the table below.

EFFECT OF RAPAMYCIN + MPA ON INTIMAL THICKENING IN INJURED ABDOMINAL AORTAS (DAY 14)

Group	Dose	Treatment Days	Intima Percent ± S.E.
Uninjured Control			0.00 ± 0.00
Untreated Injured Control			10.17 ± 2.42
Rapamycin + MPA	40/1.5 mg/kg	–3–13	0.00 ± 0.00

The results in the tables above show that rapamycin, alone or in combination with mycophenolic acid, is useful in preventing restenosis following invasive procedures that disrupt the vascular endothelial lining, such as percutaneous transluminal coronary angioplasty, vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures. These data also show that the administration of rapamycin, alone or in combination with mycophenolic acid, from 3 days pre-catheterization to 13 days post-catheterization, allowed the endothelium to heal, while preventing intimal smooth muscle cell proliferation. That intimal proliferation did not occur 31 days after administration with rapamycin, alone or in combination with mycophenolic acid, had been stopped, demonstrates that the endothelial layer had regenerated, as intimal proliferation stops after the reestablishment of the endothelial layer. The reestablishment of an intact endothelial layer was confirmed by microscopic examination of the previously catheterized arteries after removal at 44 days.

From the data above, it is particularly preferred that treatment begin with rapamycin or rapamycin plus mycophenolic acid before the procedure is performed, and that treatment should continue after the procedure has been performed. The length of treatment necessary to prevent restenosis will vary from patient to patient. For percutaneous transluminal angioplasty procedures, it is preferred that treatment be administered from 3 or more days before the procedure and continuing for 8 or more days after the procedure. It is more preferred that administration will be for 3 or more days before the angioplasty procedure and continuing for 13 or more days after the procedure. The same administration protocol is applicable when rapamycin, alone or in combination with mycophenolic acid, is used to prevent restenosis following vascular catheterization, vascular scraping, vascular surgery, or laser treatment procedures.

When rapamycin is employed alone or in combination with mycophenolic acid in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin, alone or in combination with mycophenolic acid, may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin, alone or in combination with mycophenolic acid, may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin, alone or in combination with mycophenolic acid can be administered intravascularly or via a vascular stent impregnated with rapamycin, alone or in combination with mycophenolic acid, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin, alone or in combination with mycophenolic acid, may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1–5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.001–25 mg/kg, preferably between 0.005–10 mg/kg, and more preferably between 0.01–5 mg/kg. Projected daily oral dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.005–50 mg/kg, preferably between 0.01–25 mg/kg, and more preferably between 0.05–10 mg/kg. Projected daily intravenous dosages of mycophenolic acid, when used in combination with rapamycin, would be 0.5–75 mg/kg and preferably between 5–50 mg/kg. Projected daily oral dosages of mycophenolic acid, when used in combination with rapamycin, would be 1–75 mg/kg and preferably between 10–50 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, rapamycin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

What is claimed is:

1. A method of treating a hyperproliferative vascular disease selected from the group consisting of intimal smooth muscle cell proliferation, restenosis, and vascular occlusion, wherein the intimal smooth muscle cell proliferation, restenosis, or vascular occlusion is caused by an infectious disorder, hypothermia, or irradiation, which comprises administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

2. A method of treating a hyperproliferative vascular disease selected from the group consisting of intimal smooth muscle cell proliferation, restenosis, and vascular occlusion, wherein the intimal smooth muscle cell proliferation, restenosis, or vascular occlusion is caused by vascular catheterization, vascular scraping, percutaneous transluminal/coronary angioplasty, vascular surgery, or laser treatment, which comprises, administering an antiproliferative effective amount of rapamycin to said mammal orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

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